Volume 48, No. 11
November 1959

BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

TRANSLATED FROM RUSSIAN

CONSULTANTS BUREAU ENTERPRISES, INC.

ANNOUNCING

a new journal in the field of
Biological Sciences
—translated from Russian

Antibiotics

(Antibiotiki)

Translated and published with the assistance of
the following companies in underwriting a
portion of the expenses involved:

ELI LILLY AND CO., INC.
THE UPJOHN COMPANY
BRISTOL LABORATORIES, INC.
CHAS. PFIZER AND CO., INC.

Published Bimonthly
Starting with No. 1, 1959

ANNUAL SUBSCRIPTION: $25.00

CONSULTANTS BUREAU, INC.
227 WEST 17TH STREET, NEW YORK 11, N. Y

Volume 48, No. 11
November 1959

BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

A publication of the Academy of Medical Sciences of the USSR

Year and issue of first translation:
Vol. 41 No. 1 *January 1956*

Annual Subscription	*$20.00*
Single Issue	*10.00*

TRANSLATED FROM RUSSIAN

Copyright 1960.

CONSULTANTS BUREAU ENTERPRISES, INC.
227 W. 17th St., NEW YORK 11, N.Y.

EDITORIAL BOARD OF

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

V. N. Chernigovskii (Editor), A. Ya. Alymov, N. N. Zhukov-Verezhnikov (Acting Editor), S. Ya. Kaplanskii, G. N. Kryzhanovskii (Secretary), M. N. Livanov, L. D. Liozner, I. N. Maiskii, V. A. Negovskii, V. V. Parin (Acting Editor), V. A. Sanotskii, A. A. Solov'ev, L. L. Shik.

The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

Note: The sale of photostatic copies of any portion of this copyright translation is expressly prohibited by the copyright owners.

Printed in the United States of America

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

Volume 48, Number 11 November, 1959

CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
The Effect on Cardiovascular Reflexes of Stimulating Pericardial Receptors. Communication IV. The Effect of Forced Respiration on Reflexes Originating from Stimulation of the Pericardial Chemoreceptors. B. S. Kulaev.	1311	3
Effect of Respiratory Changes on the Degree of Arterial Oxygen Saturation. N. V. Sanotskaya	1316	8
Human Postural Movements in Increased and Reduced Gravitational Fields. V. S. Gurfinkel', P. K. Isakov, V. B. Malkin and V. I. Popov	1320	12
Olfactory Sensitivity at Rest and During Sound Stimulation. R. Z. Amirov	1326	18
The Effect of Early Partial Decortication on Visual and Auditory Conditioned Reflexes in Chickens. O. E. Ponomareva	1330	22
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
Restoration of Vital Function in Monkeys After Bleeding to Death During Hypothermia. V. A. Negovskii, V. I. Soboleva, N. L. Gurvich, K. S. Kiseleva and Sh. S. Machavariani	1338	30
Compensatory Changes in the Kidney After Unilateral Nephrectomy. V. I. Fedenkov	1342	34
Central Nervous Changes in Experimental Tetanus and the Mode of Action of the Tetanus Toxin. Communication II. The Part Played by Spinal Mechanisms. G. N. Kryzhanovskii	1346	38
An Electrophysiological Study of Different Parts of the Nervous System in Shock. V. S. Sheveleva	1351	43
Relation of Splenic Activity to that of Other Organs. Communication III. Effect of Diathermy Applied to Spleen and of Spleen Extracts on the Liver and Hemopoietic Organs During Pathological Conditions. L. I. Geller	1358	50
The Action of Low Temperatures and Ionizing Radiation on the Serum Proteins and Protein Fractions. S. A. Brailovskii and Yu. K. Ledentsov	1365	57
The Correlation of the Content of Sulfhydryl Groups with the Cerebroside Metabolism of the Brain in the Rabbit. M. Sh. Promyslov and M. S. Morozova	1368	59
The Role of the Colloid in the Hormone-Forming Function of the Thyroid Gland. M. F. Merkulov	1370	61
The Effect of Ribonucleic Acid and Its Hydrolyzates on the Uptake of Glycine-1-C^{14} by Proteins of Normal and Tumor Tissues. I. B. Zbarskii and N. P. Karuzina	1375	65

CONTENTS (continued)

	PAGE	RUSS. PAGE
Changes in the Ammonia and Glutamine Content of the Brain in Rats After Administration of Sodium Bromide and Caffeine and in Carbon Tetrachloride Poisoning. B. G. Gordon	1380	69
The Biological Role of Uranium in the Mammalian Body. D. P. Kladienko	1384	73
PHARMACOLOGY		
The Effect of Dibasol on the Auditory Adaptation. A. P. Velitskii	1387	77
The Effect of Salts of Copper and Manganese on the Cholinergic Systems of the Heart. V. S. Raitses and Ya. V. Ganitkevich	1391	81
MICROBIOLOGY AND IMMUNITY		
The Deliberate Cultivation of Antitumor Properties in Bacteria. L. I. Oleinik and V. N. Yagodinskii	1396	86
The Immuno-Adaptive Stage in Rats in Relation to Normal and Heterologous Cells. J. Grozdanovic and A. Puza	1400	90
ONCOLOGY		
Changes in the Immunobiological Properties of a Tumor After Passage Through a Chick Embryo. T. A. Pokrovskaya	1403	93
The Effect of Antitumor Serum on the Mitotic Activity of Neoplastic and Normal Tissues in the Mouse. S. V. Sukhorukikh	1407	97
The Effect of Late Reimmunization on the Production of Antitumor Antibodies. P. P. Filatov	1411	101
EXPERIMENTAL BIOLOGY		
The Precipitation Reaction Between the Fertilizin of the Ova and the Antifertilizin of the Spermatozoa in Polychaeta of the White Sea. L. L. Lishtvan	1414	104
The Mechanism of the Diurnal Periodicity of Mitosis. I. A. Alov	1417	107
MORPHOLOGY AND PATHOMORPHOLOGY		
Morphological Changes in the Skin in Response to External α-Irradiation. F. L. Leites	1424	113
METHODS		
A Pneumoelectric Converter for Physiological Research. V. K. Sel'tser	1430	118
Colorimetric Estimation of Thiamin by the Ninhydrin Reaction. Yu. M. Ostrovskii and M. A. Gvozdeva	1433	120
A Study of the Properdin System. Communication I. A Simplified Method of Estimation of Properdin. K. M. Rozental' and G. B. Savel'vol'f	1435	122

PHYSIOLOGY

THE EFFECT ON CARDIOVASCULAR REFLEXES OF STIMULATING PERICARDIAL RECEPTORS

COMMUNICATION IV. THE EFFECT OF FORCED RESPIRATION ON REFLEXES ORIGINATING FROM STIMULATION OF THE PERICARDIAL CHEMORECEPTORS

B. S. Kulaev

From the Laboratory of General Physiology (Head – Active Member of the AMN SSSR V. N. Chernigovskii) of the Institute of Normal and Pathological Physiology (Director – Active Member of the AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Received May 8, 1959. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

It has been shown previously [7, 8] that in most experiments a weak chemical stimulation of the pericardial receptors causes tachycardia and increased arterial pressure; stronger stimulation causes bradycardia and a reduced blood pressure. There is always a hyperpnea, which increases in proportion to the strength of the stimulation. It has been suggested that the two opposite effects were performed by different rates of afferent impulses from the cardiac and bulbar centers [5, 7, 8]. After denervation of the sinocarotid zone, both strong and weak chemical stimulation of the pericardial receptors caused only a constriction of the blood vessels, while the heart itself continued to give the two reactions as before [9]. It may be concluded that the regulatory effect on the heart is determined by the number of impulses reaching it at any one time [15, 18], whereas the vascular reaction is determined in the vasomotor center by either excitation or inhibition of the efferent elements by afferent impulses arriving at a high rate. In order to confirm this suggestion, it was important to develop an experiment on a cat which would allow increased afferent stimulation of the bulbar centers, so that any stimulation, even a weak one, applied to the pericardium would bring about only a depressor reaction, as was observed in rabbits [16, 17].

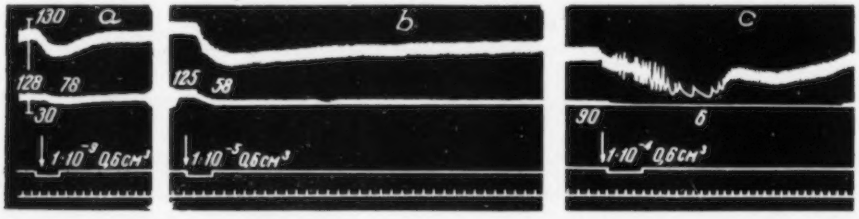


Fig. 1. Reduction in arterial pressure and bradycardia in response to stimulation of the pericardial receptors with different concentrations of nicotine. Cat. Urethane. Forced respiration. Value of the curves, from above downwards: pressure in the carotid artery (mercury manometer), ditto (membrane manometer), stimulus marker (⊙– application of nicotine), time marker (5 seconds); a) reflex induced by injection of 0.6 ml of $1 \cdot 10^{-9}$ nicotine solution; b) ditto, 0.6 ml of $1 \cdot 10^{-5}$ nicotine; c) ditto, 0.6 ml of $1 \cdot 10^{-4}$ nicotine. Figures above the membrane manometer trace indicate heartbeats per minute.

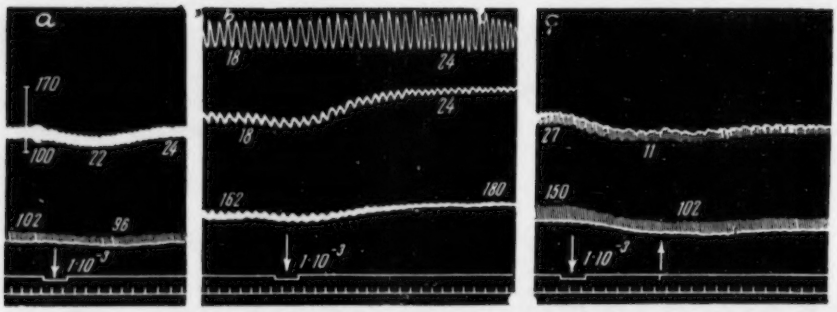


Fig. 2. Cardiovascular changes induced by stimulation of the pericardial receptors with $1 \cdot 10^{-3}$ nicotine solution. a) forced respiration, b) natural respiration, and c) forced respiration resumed; ↓– nicotine washed out. Curves, from above downwards, for a and c: pressure in carotid artery (mercury manometer); ditto (membrane manometer), stimulus marker, time marker (5 seconds); for b) uppermost curve – respiration, figures below the curve of arterial pressure indicate the number of arterial pressure waves of the second order per minute.

We have succeeded in developing just such an experiment on cats, by using artificial respiration which must evidently induce powerful stimulation of the mechanoreceptors of the lungs.

Afferent effects from the lungs on the cardiovascular system have been investigated repeatedly. Weak electrical stimulation of the pulmonary branches of the vagus nerves induces tachycardia and raises the arterial pressure [19, 29, 30], whereas strong stimulation causes bradycardia and a reduced blood pressure [19, 21, 30]; a small increase in the inspired volume of the lungs causes tachycardia and increased arterial pressure, while a greater increase causes bradycardia and a reduced blood pressure [19, 27, 30, 31]. Reducing the pressure in the pulmonary vessels causes tachycardia and a pressor reaction, while increasing it causes bradycardia and a fall in blood pressure [10, 11, 20, 22, 32]. Weak chemical stimulation of the mediastinal pleura, where the receptors are most concentrated than in any other portion of the respiratory system [14, 28], causes tachycardia, raised blood pressure, and hyperpnea, while weak stimulation causes bradycardia, reduced blood pressure, and inhibition of respiration [4].

The afferent pathways of all these reflexes and the efferent routes of cardiac and pulmonary reflexes run through the vagi and have their center in the medulla, while the efferent pathways of the vasomotor reactions run in the sympathetic system. This means that reflex effects from the lungs on the cardiovascular system are closely related both functionally and morphologically to reflexes from the pericardium and sinocarotid zone. It might be possible, therefore, that the conditions of the vasomotor center might be considerably modified by changing the rate of flow of afferent pulmonary impulses.

In all, 60 experiments were carried out.

The method of investigation was described in part I of this series [7].

RESULTS

In all experiments in which forced ventilation of the lungs was used, application of nicotine to the pericardial receptors caused only a depressor reaction and bradycardia, whatever the concentration (Fig. 1, a, b, c).

The transition from the pressor reaction with natural breathing [5, 7, 12] to the exclusively depressor reaction with artificial respiration takes place very rapidly. Fig. 2 shows the effect of three successive stimulations with $1 \cdot 10^{-3}$ nicotine solution applied at different time intervals. The first and third stimuli were applied during forced respiration, and caused a depressor reaction and bradycardia (Fig. 2, a, c); the second stimulation, applied between the first and the third, during the interval with natural respiration, caused a pressor reaction and tachycardia (Fig. 2, b).

In cases when animals with previous denervation of the carotid sinus were transferred to artificial respiration, and when only very intense pressor reactions were observed [9], the transition lasted somewhat longer, and it was possible to observe the intermediate stages. These can be seen in Fig. 3, where a series of kymograms from such an experiment are shown.

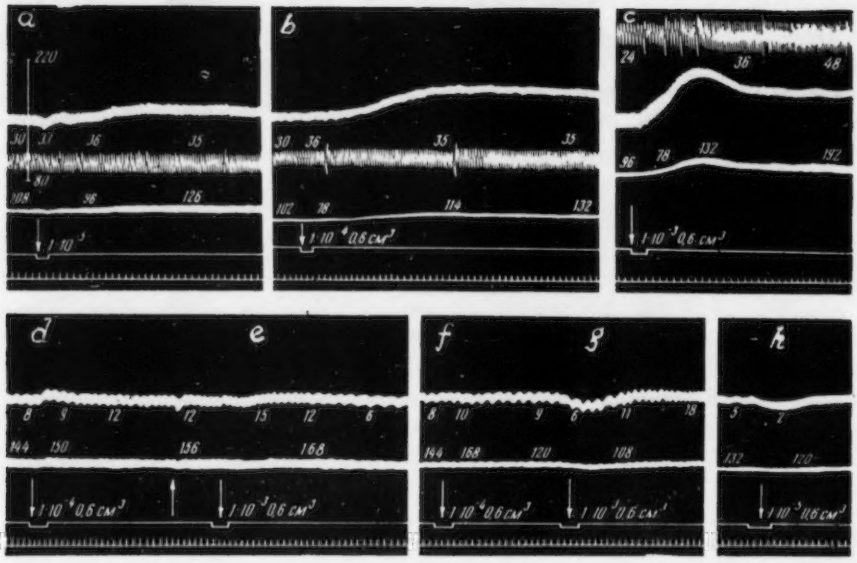


Fig. 3. Changes in the cardiovascular reactions in response to stimulation of the pericardium with various concentrations of nicotine during transition from natural (a, b, c) to forced respiration (2-3) in an animal with previous denervation of the sinocarotid reflexogenous zone. Curves, from above downwards, for a and b: arterial pressure in carotid artery (mercury manometer), respiration, arterial pressure (membrane manometer), stimulus marker (↓ –injection of nicotine), time marker (5 seconds), for c – respiration, arterial pressure in carotid artery (mercury manometer), ditto (membrane manometer), stimulus marker (↓ – injection of nicotine), time marker (5 seconds), for d, e, f, g, h curves as in Fig. 2,c.

This transition from pressor to depressor reactions can be observed also in animals with intact carotid sinuses, but in this case the transition occurs much more quickly, and the depressor reaction and bradycardia are very marked.

It is important to note one typical phenomenon shown very clearly in Figs. 2 and 3. Immediately after the transition to forced respiration, second order blood pressure waves scarcely differing from those due to natural respiration can be clearly seen. In some experiments, at low natural rates of respiration (18 per minute) these second order waves were more frequent and smaller in amplitude than those recorded with natural respiration (Fig. 2), while in other experiments when the natural breathing rate was higher (30 per minute), the waves were smaller in amplitude and lower in frequency (Fig. 3). However, in both cases the waves did not coincide with the rhythm of the forced respiration, but had their own rhythm which evidently reflected the activity of the respiratory center as affected by pericardial stimulation: the frequency increased in the first few minutes after the onset of forced respiration (see Fig. 3, d, e), it was slowed subsequently (see Fig. 2, a, c and Fig. 3, h), and at the intermediate stages it was increased in response to weak and reduced in response to strong stimulation (Fig. 3, f, g). During this time, the rate of forced respiration was kept constant. With natural respiration, chemical stimulation of the pericardial receptors caused only an increase in the rate of respiration [5, 7] and an increase in the second order waves (Fig. 2, b).

In experiments in which action potentials from the vagus were recorded on a cathode ray oscillograph, we succeeded in showing that the discharge rats from the lungs increases several fold, and that the increased frequency is maintained during forced breathing. The increased rate of flow of impulses following reflex hyperpnea during natural breathing did not as a rule reach such a high value.

Our experiments have shown that cats in whom artificial respiration was applied represent the opposite model to that which we obtained with previous denervation of the sinocarotid zone. Those in whom forced respiration was applied showed a considerable increase in the flow of afferent impulses in the vagi, as well as depressor reactions and bradycardia in response to pericardial chemical stimulation at all intensities.

The possibility that the changed gaseous constitution of the blood might have some effect on the reactions has been discussed [2], and was convincingly refuted by I. de Burg Daly and M. Scott [23]. However, these authors

did not take into account the fact that stabilization of impulses from the lungs by forced respiration is the result of increasing the rate to a value higher than that which occurs in reflex hyperpnea and maintaining it for a longer period. It is percisely these afferent impulses which in our experiments brought about the controlled condition of the bulbar and cardiac centers which enabled any cardiac receptor stimulation to cause a fall in blood pressure, bradycardia, and inhibition of the respiratory center (insofar as the latter could be inferred from reduction in the frequency of the second order waves). Reflex hyperpnea during natural respiration cannot, according to our results, be considered to be a certain cause of a secondary reflex tachycardia, because strong stimulation evoked both hyperpnea and bradycardia; evidently, the same thing occurs on stimulation of the carotid chemoreceptors [3].

Stimulation of the carotid chemoreceptors by hypoxia during natural respiration as a rule brings about the same changes as does stimulating them with low concentrations of drugs [1, 3, 26], whereas high concentrations of drugs cause a high rate of afferent impulsation, a far greater hyperpnea, bradycardia, and a reduction of arterial pressure [3]. Therefore, the relationship between the cardiac reaction and the intensity of reflex hyperpnea as demonstrated by I. de Burg Daly and M. Scott is valid only for the weakest stimulation of the carotid chemoreceptors, and corresponds to what was found by W. Douglas and has co-workers [24, 25], during the transition from weak to moderate electrical stimulation of the sinocarotid nerves; however, it does not apply to stronger pharmacological action on the chemoreceptors [3] or to strong electrical stimulation of the sinus nerves [24, 25].

In studying the reflex effects on heart rate of electrical stimulation of the branchial nerves in fish [6], we have found that weak stimulation of any branchial nerve causes tachycardia, and a stronger stimulation, bradycardia. Simultaneous stimulation of any two of these nerves causes a bradycardia even when stimulation of either of them separately at the same intensity caused a tachycardia. Similar results were obtained by M. G. Udel'nov and his co-workers [15, 18] on frogs by stimulating different afferent nerves and reflexogenous zones. They showed that the involvement of a comparatively small number of vagus fibers supplying the heart causes tachycardia and that bradycardia results when a large number of fibers are involved.

I. de Burg Daly and M. Scott [23] imitated hyperpnea by an extremely high rate of forced respiration; they induced what was evidently a partial inhibition of the bulbar vagal centers, so causing a tachycardia.

Increase of afferent impulses from the lungs during forced breathing causes a fall in arterial pressure to occur in response to any chemical stimulation of the pericardial receptors, and this results not only from a bradycardia, but also from an inhibition of the vasomotor center through summation of the afferent effects from both lungs and heart.

The changes which we have found in the second order arterial pressure waves indicate that the same process applies torespiratory regulation. The bradycardia, reduction in arterial pressure, and apnea, form what is known as the Betsol'd-Yarish triad, which from our results should be regarded as the result of summation in the bulbar centers of the increased flow impulses from the thoracic receptors, and should not be attributed to specific depressor influences.

Thus, at a certain optimal level of the combined afferent inflow, weak stimulation of the caridac receptors causes an increased arterial pressure and tachycardia, while a strong stimulation causes a fall in pressure and bradycardia. Reduction of the general afferent inflow level through a reduction of the contribution of any of the thoracic reflexogenous zones causes an increase in arterial pressure and has the effect that any stimulation of the cardic receptors will produce only pressor reactions. Increase in the afferent flow rate will produce the opposite effect.

SUMMARY

Acute experiments were performed on cats with the employment of artificial respiration. In these animals the chemical stimulation of pericardial receptors provoke only depressor reactions of the arterial blood pressure, bradycardia and inhibition of waves of the second order.

The pressor reactions reappear after the restoration of natural respiration. Such relations are also observed in animals with previously denervated sinocarotid zones.

Proofs are given of the fact that the depressor reaction and bradycardia during artificial respiration are due to the increased flow of pulmonary afferent impulses.

LITERATURE CITED

[1] K. Geimans and D. Kord'e, The Respiratory Center. Leningrad, 1940.•

[2] É. Gelhorn, The Regulatory Function of the Autonomic Nervous System,• Moscow, 1948.

[3] V. V. Zakusov, Fiziol. zhurn. SSSR, 1938, volume 25, issue 3, p.569.

[4] D. A. Kocherga, Byull. éksptl. biol. i med., volume 39, No.2, p.7 (1955).

[5] B. S Kulaev, The Role of The Chemoreceptors of the Pericardium in the Regulation of Blood Circulation and Respiration,• Dissertation, Moscow, 1954.

[6] B. S. Kulaev, Byull. éksptl. biol. i med., volume 45, No.4, p.8 (1958).••

[7] B. S. Kulaev, Byull. éksptl. biol. i med., volume 45, No.6, p.17 (1958).••

[8] B. S. Kulaev, Byull. éksptl. biol. i med., volume 46, No. 10, p.23 (1958).••

[9] B. S. Kulaev, Byull. éksptl. biol. i med., volume 46, No.12, pp. 8-14 (1958).••

[10] V. V. Parin: Transactions of the Sverdlovsk Medical Institute and Scientific Research Institutes of the Oblast Roads Division.• Severdlovsk, 1941, collection 15, pp. 3-87.

[11] V. V. Parin, The Role of the Pulmonary Vessels in Reflex Regulation of the Circulation,• Moscow, 1946.

[12] I. M. Rodinov, Moscow UniversityBulletin, Moscow, No. 3, p.95 (1957).

[13] P. A. Spiro, Report of the Novorossiisk Unversity. Odessa, 1874, volume 15, No.1

[14] I. V. Torskaya, Med. zhurn., issue 3, p.75 (1952).

[15] M. G. Udel'nov, The Structural and Functional Basis of the Inhibitory Action of the Nervous System and the Nature of the Process of Cardiac Inhibition.• Dissertation. Moscow, 1955.

[16] V. N. Chernigovskii, in book: Neurohumoral Regulation in the Activity of Organs and Tissues,• Leningrad, 1941, p.54.

[17] V. N. Chernigovskii, The Afferent Systems of the Internal Organs,• Kirov, 1943.

[18] N. L. Yastrebtsova and M. G. Udel'nov, in book: Problems of the Pathology and Physiology of the Heart.• Moscow, 1955, p.140.

[19] G. V. Anrep, W. Pascual and R. Rössler., Proc. Roy. Soc., 1936, Bd. 119, p.191.

[20] D. M. Aviado Jr., T. H. Li, W. Kalow, C. F. Schmidt, G. L. Turnbull, G. W. Peskin, M. E. Hess and A. I. Weiss, Am. J. Physiol., 1951, v.165, p.261.

[21] T. G. Brodie and A. E. Russell, J. Physiol., 1900, v.26, p.92.

[22] I. de B. Daly, G. Ludany, A. Todd and E. B. Verney, Quart. J. exp. Physiol., 1937, v.27, p.123-146.

[23] I. de B. Daly, and M. J. Scott, J. Physiol., 1958, v.144, p.148.

[24] W. W. Douglas, J. R. Innes and H. W. Kosterlitz , J. Physiol., 1950, v.111, p.215-230.

[25] W. W. Douglas and W. Schauman , J. Physiol. 1956, v.132, p.173.

[26] U. S. von Euler, G. Liljestrand and Y. Zotterman, Scand. Arch. Physiol., 1939, Bd. 83, H. 1, 2, 3.

[27] E. Hering, S. B. Akad. Wiss. Wien, 1871, Bd. 64, S.333.

[28] R. J. Honjin, Comp. Neurol., 1956, v.105, p.3.

[29] P. Kanoll, Jahrb. Lotos, Prague, 1881, Bd. 2, S.1-34.

[30] E. von Saalfeld, Pflug. Arch. ges. Physiol., 1933, Bd. 231, S.33.

[31] E. Von Saalfeld, Pflug. Arch. ges. Physiol., 1933, Bd. 231, S.724-728.

[32] H. Schwiegk, Pflug. Arch. ges. Physiol., vol. 236, 206 (1935).

•In Russian.

••Original Russian pagination. See C. B. translation.

EFFECT OF RESPIRATORY CHANGES ON THE DEGREE OF ARTERIAL OXYGEN SATURATION

N. V. Sanotskaya

From the Laboratory of the Physiology and Pathology of Respiration and Circulation (Head – Corresponding Member of the AMN SSSR M. E. Marshak) of the Institute of Normal and Pathological Physiology (Director – Active Member of the AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Received April 13, 1959. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

For normal oxygenation of the pulmonary blood, there must be a close correlation between ventilation and blood flow. Disturbance of this correlation is the principal cause of arterial hypoxia in health or disease, as is shown by many experimental and clinical studies [1-7]. However, in studying hypoxia, insufficient attention has been paid to the relative importance of changes in ventilation and in the lesser circulation. Little work has been done either on the mode of action of different disturbances of pulmonary ventilation on the degree of oxygenation of the blood; nevertheless, it is extremely important to have this information in order to determine the causes of hypoxia in different conditions.

The object of the present investigation has been to study the effect of respiratory changes (pulmonary ventilation, rate, rhythm, and amplitude of respiratory movements) on the degree of blood oxygen saturation.

METHOD

In all, 58 experiments on rabbits, cats, and dogs were made, using acute preparations under urethane anesthesia.

Records were made of respiratory movements, arterial pressure, and blood oxygen saturation; in some of the experiments pulmonary ventilation was also recorded.

To determine changes in the degree of arterial blood oxygen saturation, we used the photoelectric recorder proposed by M. E. Marshak, which is a modification of that described by Kramer [8]; the recorder was applied directly to the artery.

Different methods of changing the respiration for the purpose of including hypoxia were used, and these included stimulation of the phrenic nerve (either the whole nerve or its central or peripheral ends), stimulation of the vagus, introducing ammonia vapor into the lungs, and other methods. Also, some experiments were tried using artificial respiration, and varying the respiratory rate, depth, and rhythm.

RESULTS

It was found that actions causing a reduction in the depth or frequency of respiration or a reduction in pulmonary ventilation were always accompanied by a marked reduction in the degree of arterial blood oxygen saturation. Fig. 1, a shows the response to stimulation of the upper respiratory pathways by the vapor from a 30% ammonia solution. There was an arrest of respiration (followed by small occasional respiratory movements), a marked increase in arterial pressure, and a considerable reduction in the arterial blood oxygen saturation, which was reduced more than by inspiring a mixture containing 9-10% of oxygen.

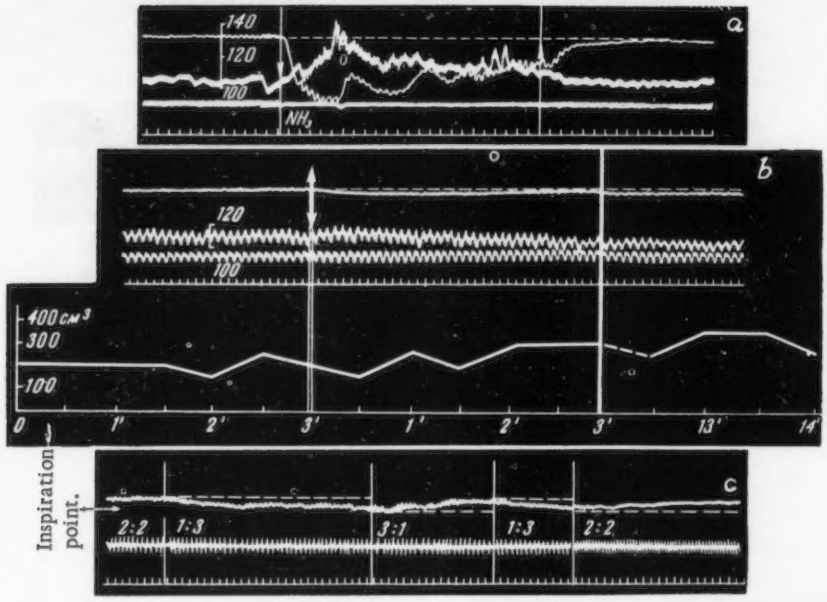


Fig. 1. Changes in the degree of arterial blood oxygen saturation. a) experiment on rabbit, 2.7 kg, 6/6, 1957. Reduction to stimulation of the upper respiratory pathways by vapor from a 30% ammonia solution (beginning and end of stimulation shown by arrows). Curves, from above downwards: degree of arterial blood oxygen saturation, blood pressure, respiration (pneumogram), time marker (5 seconds); b) experiment on dog, 6 kg. 1/20, 1958. Reaction to sectioning the two phrenic nerves. Curves, from above downwards: arterial blood oxygen saturation, blood pressure, respiration, time marker (5 seconds), arterial ventilation in cm^3 per 30 seconds. Thick vertical line — break in recording; c) experiment on cat, 2 kg, 11/28, 1957. Reaction to forced changes in the respiratory rhythm (animal immobilized by injection of 10 mg/kg diplacin; artificial respiration). Vertical lines indicate transition to respiration with a changed ratio of duration of inspiration (first figure) and expiration (second figure), as controlled by strokes from metronome. Curves, from above downwards: degree of arterial blood oxygen saturation, respiration, time marker (5 seconds). Dotted line shows initial blood oxygen saturation level.

A reduced oxygen saturation may occur without a reduction in pulmonary ventilation or, if such a reduction has occurred, may be produced after the rate has already regained its original value or surpassed it. This is found, for instance, on sectioning the phrenic nerves (see Fig. 1, b), when there is an immediate reduction in arterial blood oxygen saturation, but none in pulmonary ventilation; on the contrary, the latter increases somewhat due to increased thoracic respiration. Hypoxia here must be explained as being due to uneven ventilation of different parts of the lungs, since when the diaphragm is put out of action, the lower lobes of the lungs are insufficiently ventilated, while the blood supply to them is maintained. This shows that a high general level of pulmonary ventilation is not the only circumstance necessary for normal oxygenation of blood in the lungs. It is very important that there should be an even ventilation of all parts of the lungs and a corresponding blood supply to these parts.

Failure of coordination, unevenness, and arrhythmia of the respiratory movements may play an important part in the development of hypoxia. Figure 2 shows respiration disturbances occurring during the operation for excision of a lung in man in response to manipulations on the root (on the pulmonary vessels and particularly on the bronchus). It has been shown that it is precisely at these stages in the operation that marked reduction in arterial blood oxygen saturation occurs [5]. In unilateral open pneumothorax, when there is a redistribution of blood supply in the lesser circulation, the greater part flowing to the second (functional) lung, these respiratory disturbances no doubt play an important part in the development of hypoxia.

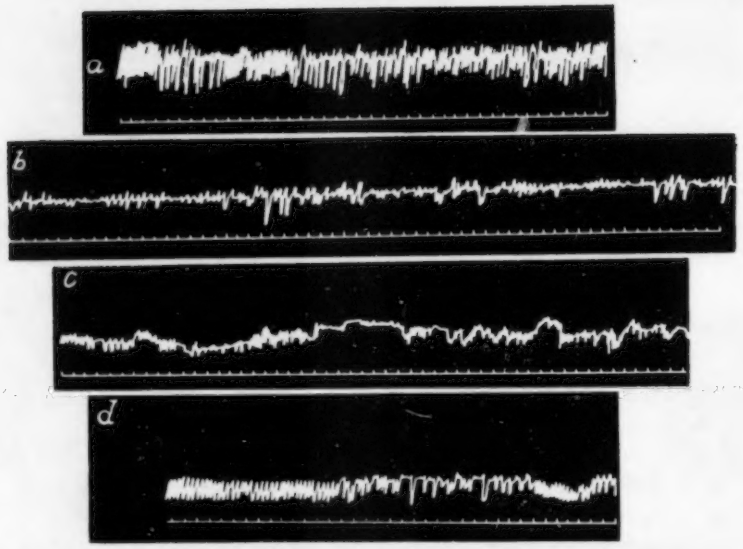


Fig. 2. Respiratory disturbances observed during pulmonary excision in man (pneumograms): a) during anesthesia of lung root; b) while dividing the pulmonary vessels; c, d) during manipulation of the bronchus. Time marker — 1 second.

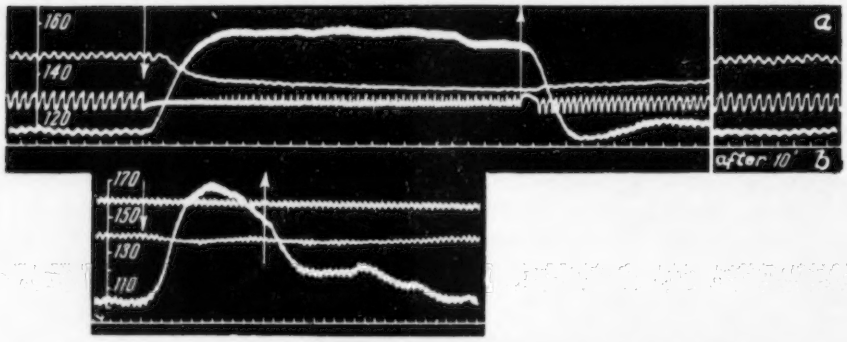


Fig. 3. Changes in the degree of arterial blood oxygen saturation during (a) natural and (b) artificial respiration. Experiment on dog, 9 kg, 1/30,1958. Reaction to simultaneous stimulation of central ends of vagal and cervical sympathetic nerves. Curves, from above downwards: for a) degree of arterial blood oxygen saturation, respiration, blood pressure; for b) respiration, degree of arterial blood oxygen saturation, blood pressure. Arrows indicate onset (↓)and end (↑)of stimulation; thick vertical line indicates break in recording. Time marker — 5 seconds.

Hypoxia can also be observed in response to changes involving the structures concerned in the act of respiration, and when respiratory rhythm is impaired.

Fig. 1, c illustrates changes in arterial blood oxygen saturation occurring during artificial respiration, when the respiratory rhythm, i.e., the ratio of the period of inhalation to exhalation, is arbitrarily changed while maintaining the rate of ventilation constant. An increase in the duration of the inspiration is accompanied by an increased blood oxygen saturation, while reducing the duration of the inspiration and correspondingly increasing that of the expiration leads to a reduction in blood oxygen.

In order to determine the effect of circulatory changes induced by the measures applied by us during the experiment on arterial blood oxygen saturation, other experiments using artificial respiration were carried out. It was found that when respiratory changes were concluded by using artificial respiration, changes in blood oxygen were either absent or much less marked (Fig. 3). This shows that respiratory changes played the principal part in the hypoxia induced by the measures which we applied.

Thus, in our investigations, the arterial hypoxia developed through disturbance of the normal relationship between pulmonary ventilation and blood supply, and was due to inadequate ventilation of different parts of the lungs. The experiment also showed that inadequate pulmonary aeration may take place not only through a fall in the general ventilation level, but also through uneven ventilation of different parts of the lungs, as occurs with discoordinated respiratory movements.

SUMMARY

Acute experiments were performed on rabbits, cats and dogs under urethane anesthesia. The author studied the effect of respiratory changes (pulmonary ventilation, tempo, rhythm, amplitude of respiratory motions) on the degree of arterial blood saturation with oxygen.

As shown by these investigations, the influences provoking the changes of conditions of pulmonary ventilation are associated with the appearance of arterial hypoxia. Insufficient pulmonary aeration may occur when the total level of the pulmonary ventilation is decreased, as well as in irregular ventilation of different portions of the lungs in discoordination of respiratory motions.

LITERATURE CITED

[1] A. M. Kulik, Byull. éksptl. biol. i med., volume 35, No. 4, pp. 16-20 (1953).

[2] M. E. Marshak, Problems of Tuberculosis,• 1951, No. 4, pp. 8-14.

[3] M. E. Marshak, Recent Advances in Biology,• 1953, volume 36, issue 2 (5), p. 209.

[4] N. N. Savitskii, Oxygen Therapy,• Leningrad, 1940.

[5] N. V. Sonotskaya, Byull. éksptl. biol. i med., volume 36, No. 8, p. 31 (1953).

[6] A. A. Tregubov, Impairment of the Respiratory Function of the Blood in Various Pathological Processes,• Leningrad, 1947.

[7] B. S. Shershevskii, Blood Gases and the Basic Forms of Nonspecific Diseases and in Damage to the Respiratory Organs.• Dissertation, Leningrad, 1948.

[8] K. Kramer, Ztschr. Biol., 1935, Bd. 96, N 1, S. 61.

• In Russian.

HUMAN POSTURAL MOVEMENTS IN INCREASED AND REDUCED GRAVITATIONAL FIELDS

V. S. Gurfinkel', P. K. Isakov, V. B. Malkin and V. I. Popov

From the Institute of Experimental Biology and Medicine of the Siberian Department of the USSR Academy of Sciences (Director – Professor E. N. Meshalkin), Novosibirsk

(Received May 30, 1959. Presented by Active Member of the AMN SSSR V. V. Parin)

The reaction of man and animals to partial or complete weightlessness is an important problem in aviation and cosmic medicine. It is important to study the physiological changes occurring during the transition periods, when an increase in apparent weight is followed by a partial or complete loss of weight. Changes in the physiological reactions occurring during weightlessness following an acceleration, or during the reverse change, may show certain special features. This is known from the fact that two conditions may coincide, when the after-effect of the preceding stimulus and the development of compensatory reactions to the anticipated stimulus may both occur together.

The effects of weightlessness on the human organism, and on motor reactions in particular, were examined in a general way at the end of the last century by K. E. Tsiolkovskii [1], who was the founder of the science of astronautics; he thought that during complete weightlessness in man there would be a disturbance of coordination of movements, and that various illusory sensations might develop. He thought that after a certain period of weightlessness, the human subject would become adapted to it.

Experimental studies on the effects on the human organisms of short weightlessness periods have been undertaken only in recent years [2, 3, 4, 5, 6]. It has been shown that short periods of weightlessness in man and animals following periods of increased weight cause no marked respiratory or circulatory disturbances.

In the researches of Henry [3] and Beck [2] it was found that both in man and animals (mice, turtles), there was a disturbance of coordination of movements followed by subsequent adaptation. The fact that during weightlessness, as Beck [2] showed, the visual system plays an important part in the maintenance of orientation in space, favored the view that severe proprioceptive disturbances may occur.

The aim of the present work has been to study the coordination of movements during short alternating periods of increased and decreased weight.

In future, instead of talking about increased weight (when there is an increase in load), or of its reduction (during conditions when weight is reduced), for convenience we shall use the concept of " gravitational change". This must be understood as applying to the conditions of our experiments.

METHOD

The experiments were carried out in the long elevator of Moscow State University. It was possible to alter gravitation from 0.5 to 1.5 g. In some experiments, for short periods, it could be reduced to 0.3 g, and raised to 2 g. The period of reduction varied from 2 to 3 seconds.

Seven practically healthy persons took part in the different experiments, and four of these were the authors of the present article.

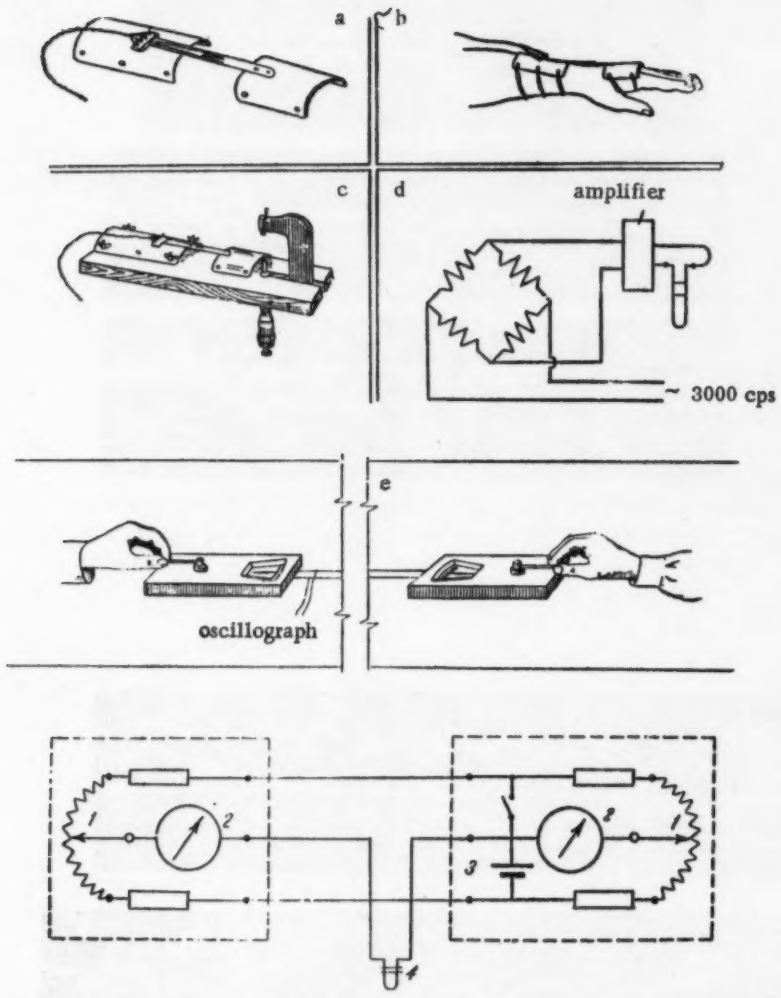


Fig. 1. Tremorgraph. a) General view, b) attachment to arm, c) calibration device, d) electrical circuit, e) device for studying coordination of voluntary movements, f) circuit of the device: 1) Potentiometer, 2) microammeter, 3) voltage supply for bridge, 4) string oscillograph.

We studied changes in the muscle while maintaining a given relative position of the arms. This method was chosen because, under changed gravitational fields, the moment of a muscular force may not be adequate to overcome the moment due to the force acting on any one section of the limb.

Starting with the frequently expressed idea that under reduced gravitation some impairment of muscle tone inevitable takes place due to alterations to the otolith apparatus, we also studied the equilibratory reactions of people in a standing position.

The third index of motor coordination studied was that of voluntary movements made in directing an arrow on a test apparatus to a certain defined position.

During the experiment, the experimenter arbitrarily and without warning to the subject caused alteration to the system which the subject had to counteract as rapidly as possible.

In this way it was possible to study the rate and precision of movements similar to those carried out by men at work.

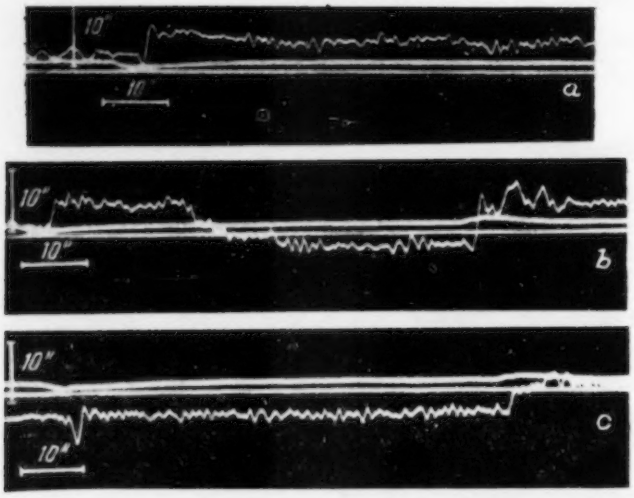


Fig. 2. Curve showing changes in the position of the hand relative to the forearm (a); b) ditto when hand is loaded with 4 kg weight; c) ditto after 10 tests. Curves, from above downwards: position of hand, change in gravitation, zero line.

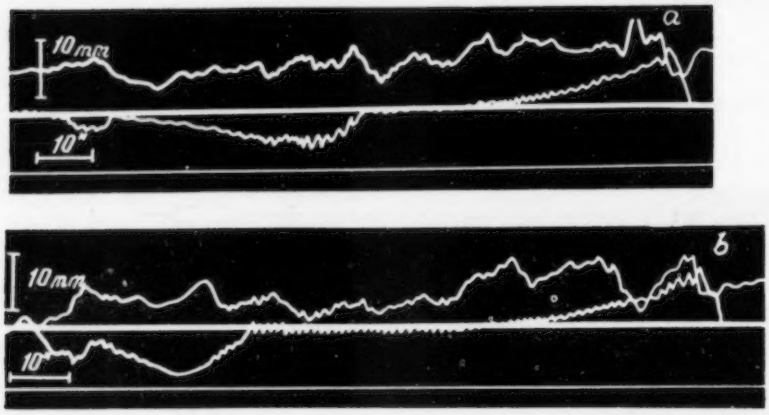


Fig. 3. Stabilogram with eyes opened (a) and with changed gravitation, b) ditto with eyes closed.

All movements were recorded by special electrical apparatus.

The relative position of the different sections of the arm were recorded by a tensiometric angle recorder, whose construction is shown in Fig. 1. Two metal platforms are connected with each other by an electric flat steel spring (Fig. 1, a). Flexion of this spring is recorded by means of a wire strain gauge attached to the spring. The gauge is included in a bridge circuit (Fig. 1, d). Recording is made on a string oscillograph. Angular indications are calibrated by a micrometer which flexes the spring through a known angle (Fig. 1, c).

The apparatus is attched by means of rubber bands to two adjacent sections of the limb (Fig. 1, b).

Records were made of the radiocarpal angle with the arm extended forward. Some of the measurements were made with loads of 2 or 4 kg added to the wrist.

Eqilibratory reactions were studied by recording the displacement of the center of gravity of the body. This was done by means of a stabilograph. The apparatus used yet another channel to record the weight of the subject, which was determined in our experiments in terms of acceleration.

The records were made with the subject in 3 positions: standing comfortably with eyes open, or closed, and when bending the body forward.

To study voluntary "working movements" of the arm, an apparatus was used whose general appearance and circuit are shown in Fig. 1, e and f.

Rotation of one of the potentiometers unbalances the bridge by an amount indicated by the pointer, and is recorded by the string on the oscillograph. One section is controlled by the experimenter, and the other by the subject. The experimenter may cause alterations in the degree or in the rate of change of unbalance of the bridge; the subject, correcting them with his potentiometers, tries to annul the unbalance. The oscillograph trace enables the rate and precision of the subject's motor reaction to be determined.

During the experiments, the whole apparatus was put in the elevator, together with the subject and the experimenter.•

RESULTS

During the investigations, both experimenters and subjects frequently experienced alternate increases and decreases in gravitation.

Even on the first day, after more than 20 such variations, quite clearly marked signs of discomfort, including fatigue, malaise, and headache, were experienced; subsequently these changes almost ceased to occur.

In studying the position of the hand relative to the forearm, it was noticed that in normal conditions, owing to the postural activity of the muscles, any given position can be maintained quite stably with very small deviations on either side of it (2-4 minutes of angle at a frequency of 8-10 per second). The position can still be maintained when weights are added to the hand; the only effect is to cause an increase in the amplitude of oscillation. This ability to maintain a position depends almost entirely on whether visual control is exerted or not.

When changing the value of the gravitational field during the first period of decreased weight, there is a disturbance of the position of the different parts of the limb. Thus, after 0.2-0.3 second from the beginning of the reduction in weight, there is a small movement of the hand upwards with respect to the forearm (Fig. 2, a). The hand remains in this position only a short time; after a few seconds the original position of the hand is almost completely restored. This change was better shown in the series of investigations in which the subject supported a 4 kg weight in the hand. As can be seen from the oscillogram of Fig. 2, b, during the period of reduced gravitation the position of the hand is disturbed; immediately afterwards, the restoration of weight is indicated by the return to normal of the hand position. During the increased gravitation there is again a disturbance of hand position relative to the forearm.

It must be noted that the absolute values of the hand displacements were relatively small, being as a rule not greater than 10-15 minutes of angle.

It is interesting that with many repetitions of induced hand movement by changing gravitation, the effect became less and less well shown. In some recordings it could be seen that the adaptation is greatest to a reduction in weight. This is very clearly seen in Fig. 2, c.

Records of hand movements made with the eyes either open or shut gave the same result, i.e. elimination of visual control had no noticeable effect on the maintenance of hand position.

Maintenance of the hand in a certain position is only one instant of postural activity. A highly coordinated structural activity specific for man is the maintenance of the upright bodily position.

Slight disturbance of equilibration is well known to clinicians and physiologists. Therefore, it seemed advantageous to study this reaction under conditions of altered gravitation.

• The authors would like to express their thanks to the Rector of Moscow State Unversity, Academician I. G. Petrovskii, and the head of the elevator staff, Comrade Rakhlis, for their cooperation in the experiments.

It can be seen from the curve of the displacement of the bodily center of gravity – the stabilogram – that there are continued equilibratory disturbances, due to the effect of the gravitational field of the earth, and that these are connected by reflex muscular reactions.

Closing the eyes causes an increase of 50-70% in the oscillations.

The stabilogram traces showed that when standing in a comfortable position with the eyes open, during reduced and increased weight there is a marked increase in the amplitude of the stabilogram waves. This increase is as great as that observed under normal conditions with the eyes closed (Fig. 3, a). The average increase in amplitude is 50-100%. However, it must be noted that the normal type of curve is still preserved: rapid displacements of the center of gravity occur, indicating equilibratory reactions associated with the maintenance of the vertical position.

Increase or decrease in weight caused a slightly greater increase in bodily oscillations when the eyes were open than when closed. It is important that under these conditions reflex equilibratory reactions were preserved (Fig. 3, b).

Under more complex conditions, when standing with the trunk bent forward, the stabilogram curves showed greater changes, and these occurred chiefly in the initial period of reduced weight.

The curves showing the movements of the right arm while controlling the position of the arrow showed that no essential changes in the coordination of movements occurred under conditions of changed gravitation.

From the results it may be inferred that under conditions of alternate 50% increases and decreases in gravitation, the regulation of position and movements, which is mediated principally by proprioceptor afferents, shows no essential alteration.

This conclusion is in agreement with recent physiological and morphological investigations of the different receptor formations, which have shown, on the one hand that there are many different kinds of proprioceptor, and on the other that the proprioceptors of the joints and ligaments play an important part in supplying information concerning the relative position and movements of the different parts of the body. Since under conditions of changed gravitation, this principal joint sense suffers no change, it is evident that coordination of movements would not be expected to suffer to any extent.

The signs of discoordination observed at the onset of gravitational changes are transient. They are more clearly shown when an external load is applied.

The possibility of the rapid adaptation of the motor system to considerably changed circumstances may be ascribed principally to the presence of a system of receptor control.

SUMMARY

Coordination of position and movements in man was studied under conditions of alternating short periods of increased and decreased gravitation. Investigations were held in the elevator of Moscow University. It was possible to produce changes of gravitation from 2 g to 0.3 g lasting for 2-3 seconds. Changes in the postural reactions of the whole body and of its separate parts and coordination of movements were registered on an oscillograph. Alternate gravitational increases and decreases did not provoke any significant disturbances in the coordination of bodily position or in the position of its separate parts, or any interference with motor reactions. Analysis of equilibrium reactions of the whole body and its separate rigid parts, with the eyes either opened or closed, demonstrated that the role of the visual system in controlling these reactions is not noticeably increased when gravitation is reduced. The data obtained indicated that when gravity is decreased by 50-70%, proprioceptor control of position and movements does not suffer materially.

LITERATURE CITED

[1] K. E. Tsiolkovskii, Complete Collected Works, Volume 2, Izd AN SSSR, 1956.*

[2] H. A. Beck, "Gravity changes in aircraft and ships." Journ. of the British Interplanetary Society, 1956, v.15, No. 2.

* In Russian.

[3] I. P. Henry, E. R. Ballinger, P. I. Marner and D. G. Simons, Animal Studies of the Subgravity State during Rocket Flight. The Journ. of Aviation Medicine, 1952, v.23, N.5, p.421.

[4] S. J. Gerathewohl, Comparative Studies on Animals and Human Subjects in the Gravity-free State. The Journal of Aviation Medicine 1954, v.25, N. 4, p. 412.

[5] T. Lomonaco, Comportamento delle funzioni corporee dell'unomo lanciato nello spazio. Rivista Aeronat 1955, v.31, N.6.

[6] D. G. Simons, Review ofbiological effects of subgravity and weightlessness. Jet. propulsion, 1955, N. 25, p.209.

OLFACTORY SENSITIVITY AT REST AND DURING SOUND STIMULATION

R. Z. Amirov

From the Laboratory of Electrophysiology (Head — Candidate of Medical Sciences R. Z. Amirov) Moscow Ear, Nose and Throat Institute (Director — Honored Scientific Worker, Professor V. K. Trutnev)

(Received November 26, 1958. Presented by Active Member of the AMN SSSR V. V. Parin)

Olfactory sensitivity depends on several factors. It can be influenced by training [5] and by the action of various drugs [4, 7].

The olfactory system has been studied comparatively little, owing to the difficult of controlling the olfactory stimulus.

The present article concerns olfactory sensitivity at rest and as affected by sound stimulation.

METHOD

An olfactometer of special construction was used, in which measured quantities of the odor could be introduced into a continuous air stream [1, 2, 3]. To study the effect of sound stimuli on smell sensitivity, pure tones from a sound generator, ZG-1, produced by the Gor'kovskii factory were used.

RESULTS

As a result of training, olfactory sensitivity increases by 2-3 micrometer divisions, and the threshold settles at a level characteristic of the subject. Sometimes variations of 0.01-0.02 mm in the threshold are observed, but usually, under constant conditions, no such alterations occur.

At the end of a working day, there is a small reduction in sensitivity. Elevation of the threshold usually amounts to 0.01-0.02 mm. Thus, for example, in the subject N., when smell sensitivity to meat odor was tested at 9 A.M., the threshold was 0.04 mm, while at 3.40 P.M. it was 0.05 mm.

In healthy persons, at the beginning of the investigation the threshold was higher than that established subsequently in the experiment. This phenomenon is particularly marked when the smell threshold for an odor is determined before the subject has been made familiar with it. Usually he does not perceive the odor when it is presented in a concentration 0.05-0.1 mm above threshold. Thus, subject N. (Fig. 1) during the tests failed to perceive an odor slightly above threshold (0.12 mm) at the first attempt. After a one-minute interval, he determined with certainty the appearance and disappearance of an odor of average strength (0.17 mm). Then gradually, the olfactometer was turned on at readings of 0.14, 0.12, and 0.1 mm, and each time the odor was perceived. At a micrometer reading of 0.09 mm there was no smell sensation, but after again presenting the smell at strength 0.10 mm, the subject was able to perceive it at a strength of 0.09 mm. There was a similar short period when there was no perception of the smell at a strength of 0.07 mm. At a micrometer reading of 0.05 mm, no sensation was evoked, and repeated stimulation likewise failed. The threshold in this particular case was 0.06 mm. The next threshold determination in this subject was started with an odor of moderate strength, which was accurately

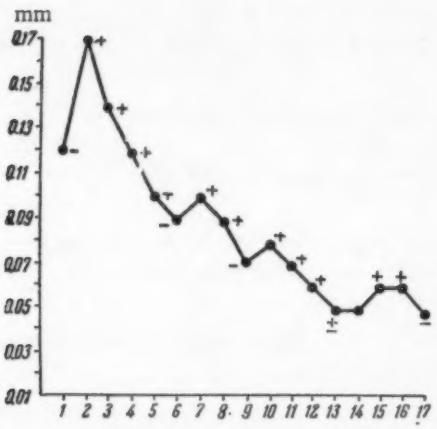


Fig. 1. Change in threshold sensitivity to an odor in the subject N. Ordinate – strength of odor of peppermint oil in relative units. Abscissa – first, second, third, etc. occasions on which odor was applied. (+) indicates subject perceives smell, (-) shows odor not perceived.

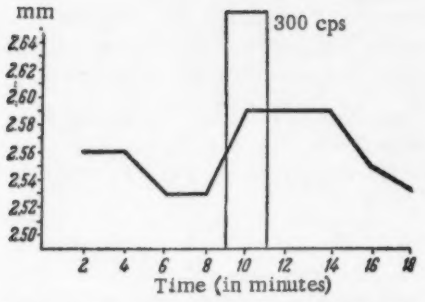


Fig. 2. Reduction in olfactory sensitivity to camphor after exposure to sound at 300 c/s. Ordinate – strength of smell in relative units. Abscissa – time of determination of threshold.

perceived, as was also an odor of strength 0.12 mm. Reducing the strength of the odor to 0.10, 0.07, 0.05, and 0.04 mm led each time to a short period during which the odor could not be perceived. The threshold was 0.03 mm.

The above example shows that at rest the olfactory threshold lies above the normal value for the subject. Therefore, when determining olfactory thresholds, it is essential to acquaint the subject with the odor beforehand.

It is known that the sensitivity of one sense organ is affected by stimuli acting on another [6]. To study the effect of sound stimulation on olfactory sensitivity, pure tones of frequencies 300, 1000, 4000, and 8000 c/s were used.

The olfactory sensitivity was determined 3 times: before applying the sound, during the sound stimulation, and finally, 3-4 times after the sound had ceased. The experiment lasted 20 minutes. Olfactory sensitivity was

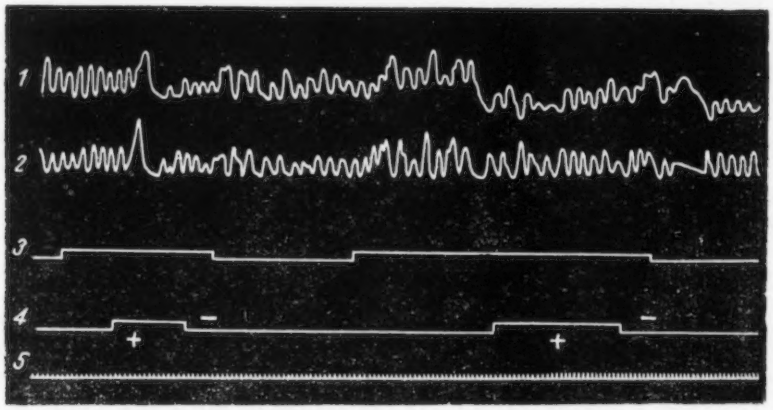


Fig. 3. Conditioned reflex adaptation to ammonia vapor odor. Curves, from above downwards: 1) thoracic respiration, 2) abdominal respiration, 3) mark indicating conditioned stimulus (sound), 4) marker indicating unconditioned stimulus (odor), 5) time marker (1 second), (+) – verbal response "it smells", (-) – verbal response "no smell".

measured every 2-3 minutes, in order to eliminate any effect due to adaptation. A more rapid method of determining smell thresholds was used: the concentrations of the vapor in the air stream were gradually raised, beginning

with subthreshold concentrations. Subjects in whom olfactory sensitivity was reduced by subthreshold stimuli were excluded from the experiments. The effect of pure tones on olfactory sensitivity to odors of camphor, ionone, peppermint oil and oil of cloves was determined.

Sensitivity to vapors of camphor and ionone following auditory stimulation at a frequency of 300 c/s was reduced in almost all cases. Reduction of the sensitivity was greatest directly after the end of the sound, and usually remained lowered until the end of the experiment. There was no change in sensitivity during the exposure to the sound.

Sound stimulation at 300 c/s raised the sensitivity to peppermint oil and oil of cloves, the greatest increase in most experiments being reached 4-6 minutes after the end of the auditory stimulus, and in some cases at the end of the experiment. The increased sensitivity to these two oils also occurred during exposure to the sound.

Thus, under the influence of a single sound of one frequency, smell sensitivity to some substances is increased, while to others it is reduced (Fig. 2).

The effect of different stimuli on olfactory sensitivity has been little studied, and the present classification of odors has been constructed on the basis of subjective experience; the above experiment with sound establishes a new principle in the physiological classification of odors based on the quantitative interaction of sense organs.

After sounds of frequencies 1000, 4000, and 8000 c/s, the olfactory sensitivity to camphor was increased. That to ionone was increased only after sounds of 4000 and 8000 c/s.

According to the extent of the olfactory sensitivity change due to the sound stimulation, it should be possible to make a more precise classification of odors and to arrange the substances in order according to the extent of the change.

At each of the frequencies 300, 1000, 4000, and 8000 c/s, the sensitivity to both clove and peppermint oils was increased. This may be because these ethereal oils are of vegetable origin and consist of several components which include a variety of odors.

Changes in olfactory sensitivity may also form the basis of conditioned reflexes. The conditioned reflex effect of sound was established in connection with the odor of ammonia solution, which is primarily a stimulus acting on the trigeminal nerve. If switching on the sound is accompanied by the odor of ammonia, then a relationship may be observed between the perception of the odor and the application of sound stimulus. With each coincidence of the sound and the odor, the latent period becomes shorter, and when there is a small lag of 4-5 seconds, the latent period becomes further reduced. The reduction of the latent period is a conditioned reflex phenomenon, since it develops as a result of the coincidence of the conditioned and the unconditioned stimulus. If the two stimuli do not coincide, the shortening of the latent period is slower and less well shown. This effect was observed in numerous investigations of the olfactory threshold, in which more than 300 measurements were made.

Under certain conditions, not only may a shortening of the latent period be observed, but an increase in length may occur. This increase may be observed after a long pause during which neither conditioned nor unconditioned stimuli are applied.

If after a series of coincidence of sound and ammonia odor, the application of the sound is reinforced by giving ammonia after an interval of 30-40 seconds, then a considerable increase in the latent period occurs. Thus, for instance, in Test No. 38-3, when the delay was made equal to 35 seconds, the latent period had a value of 18 seconds, whereas when the two stimuli were given together, or after a short interval, the latent period was 5 seconds (Fig. 3). When the latent period increased, the subject noted that the smell became weaker and scarcely noticeable. This increase in latent period must be regarded as a conditioned reflex effect of the sound on adaptation because the effect of odors is to cause an adaptation and to reduce sensitivity. After a number of coincidences, the sound becomes a conditioned stimulus which alters olfactory sensitivity. This action of the sound may occur when there is a time lag between the stimuli, and is shown both by an increase in the latent period and a reduction in the strength of the odor.

SUMMARY

Olfactory tests at rest demonstrated the following: 1) with repeated tests there was a training effect, and sensitivity rose, 2) towards the end of the day, olfactory sensitivity was reduced, 3) when a single test only is given,

the olfactory threshold is very much higher. The olfactory sensitivity to the odor of peppermint and clove oils is increased by sound at 300 c/s, while the sensitivity to camphor and ionone is decreased. The author suggests a classification of odors to be based on the effect of sound on olfactory sensitivity. A conditional reflex decrease in the sensitivity to the odor of ammonium hydroxide was found to be produced by sound stimulation.

LITERATURE CITED

[1] R. Z. Amirov, Byull. éksptl. biol. i med., volume 36, No. 8, p.76 (1953).

[2] R. Z. Amirov, 0-4 and 0-5 Olfactometers. Transactions of the State Scientific Research Ear, Nose and Throat Institute, issue 4 (1954).

[3] R. Z. Amirov. The Problem of the Role of Conditioned Reflexes in the Mechanism of Laryngo-oto-rhino Diseases and in the Study of the Higher Nervous Activity of Man. Transactions of the State Scientific Research Institute of the Ear, Throat, and Nose, issue 8 (1956).

[4] A. I. Bronshtein, Sensitization of the Sensory Organs*, Izd. S. M. Kirov Military Medical Academy, 1946.

[5] V. A. Gorovoi-Shaltan and K. Kotsyubinskii, Air Raid Defense Bulletin, No. 11 (1935).

[6] S. V. Kravkov, Interaction of Sense Organs*. Izd. AN SSSR, 1948.

[7] A. S. Mozzhukhin, The Functional Condition of the Cerebral Cortex and Sensory Organs in Penetrating Skull Wounds*. Dissertation, Military Medical Academy, 1946.

* In Russian.

THE EFFECT OF EARLY PARTIAL DECORTICATION ON VISUAL AND AUDITORY CONDITIONED REFLEXES IN CHICKENS

O. E. Panomareva

From the Department of Human and Animal Physiology (Head — Professor A. B. Kogan) Rostovskii State University

(Received May 30, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

The possibility of complete functional, and even morphological, restoration of portions of the cortex removed at an early age from dogs and cats [5, 3, 8] raises the problem of to what extent the same process may take place in animals in which the brain is less highly organized than in mammals. Birds are an interesting group to study.

The cerebral cortex of birds consists chiefly of the well developed corpus striatum. The cerebral cortex is very poorly developed, and represented only by its evolutionarily old portions and occupies a small strip covering the corpus striatum. Numerous experiments with complete excision of the cerebral hemispheres in adult birds have shown that the animals lose the power of detailed perception of the surroundings [1, 2, 4, 9, 10]. However, if only the cortical layer is removed, leaving the corpus striatum on both sides intact, then pigeons retain previously elaborated conditioned reflexes [2].

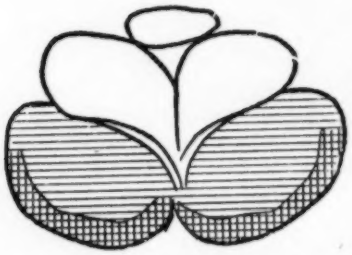


Fig. 1. Diagram of portions of cerebral cortex removed. Frontal section at boundary of middle and hind thirds; region removed in the first group shown by horizontal shading, that in the second group by vertical shading.

The present work has been carried out to investigate the possibility of restoration of the visual and auditory systems, and also to what extent morphological regeneration of the removed portions of the hemispheres may take place in young chickens.

METHOD

The experiments were carried out on "Russian White" chicks aged 6-12 days. The animals were divided into two groups. In the first group consisting of 17 birds, the posterior halves of the cerebral hemispheres, including the cortical rudiments in this region and part of the corpora striata, were removed through holes trephined on both sides of the skull. In the second group consisting of 6 birds, only the cortical rudiments lying in the superficial portion of the hemisphere over the ventricles were removed (Fig. 1). In addition, the same operations were carried out on 5 adult birds.

After 3-4 months from the time of operation, the conditioned reflexes in 28 of the operated and 14 of the control birds were studied. Conditioned reflexes were established to the opening of the feeding trough, to the light stimulus from a 75 watt 120 volt lamp, and to 500 c/s sound stimulation from a generator, all these stimuli being reinforced by feeding.

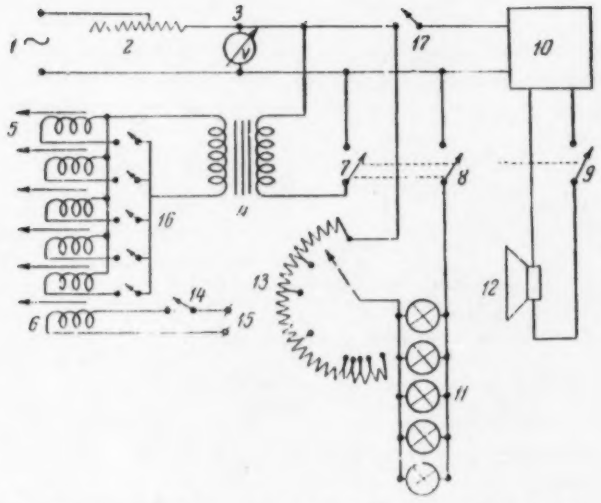


Fig. 2. Diagram of the apparatus for studying visual and auditory reflexes in chicks. 1) Main supply, 2) rheostat , 3) voltmeter, 4) step-down transformer, 5) time marker, 6) time marker, 7) common switch for markers, 8) switch for light signal, 9) switch for sound stimulation, 10) sound generator, 11) light signal lamps, 12) loudspeaker, 13) rheostat for controlling light intensity, 14) switch for time marker, 15) source of current pulses for time marker, 16) contacts of lid feeding trough, 17) switch for sound generator.

TABLE 1

Figures Showing the Formation of Conditioned Reflexes in Control and Experimental Chicks with Excised Posterior Halves of the Cerebral Hemispheres.

Indication of formation of conditioned reflexes	Kind of conditioned reflexes			
	visual		auditory	
	experiment	control	experiment	control
Number of coincidences before the first appearance of the conditioned reflex	2.4	3.9	5.5	1.5
Number of coincidences before consolidation of the reflex	11.4	8.2	6.8	2.3
Stability of the conditioned reflex (% of total number of tests to which positive responses was obtained)	70.7%	80.6%	69.9%	86.4%
Regularity of the conditioned reflex (maximal number of successive positive responses)	12.6	17.0	10.7	15.4
Latent period of the conditioned reflex (in seconds)	5.5	3.0	4.8	2.8

The experiments were carried out in 5 compartments with identical conditioned stimulation (Fig. 2). Simultaneous recordings with inkwriters were made of the application of the light or sound stimuli (conditioned stimuli) and of the opening of the lid of the feeding trough by the birds (conditioned reflex).

TABLE 2

Figures Showing the Development of Differentiations in Control and Experimental Chicks in Whichthe Posterior Portions of the Cerebral Hemispheres were Removed

Differentiated stimuli		Indication of formation of conditioned reflexes (differentiations)	Kind of conditioned reflexes (differentiations)			
			visual		auditory	
light (direct voltage applied to lamp)	sound (frequency in cps		experiment	control	experiment	control
127—60	500—3000	Number of coincidences before the development of a stable differentiation	25.5	16.9	8.4	5.4
		Stability of the differentiations effected (in %)	71.3	76.8	71.2	83.3
		Regularity (maximal number of successive differentiations carried out)	6.5	8.5	6.8	10.9
127—80	500—2000	Number of coincidences before the development of a stable differentiation	4.4	6.6	3.0	2.4
		Stability of the differentiations effected (in %)	67.5	85.0	85.0	86.0
		Regularity (maximal number of successive differentiations carried out)	5.2	7.9	5.9	6.8
127—90	500—1500	Number of coincidences before the development of a stable differentiation	6.5	7.8	1.1	2.3
		Stability of the differentiations effected (in %)	68.8	84.1	66.9	81.9
		Regularity (maximal number of successive differentiations carried out)	4.9	6.5	5.1	7.5
127—100	500—1000	Number of coincidences before the development of a stable differentiation	4.2	8.0	2.1	3.6
		Stability of the differentiations effected (in %)	72.0	81.1	71.9	82.3
		Regularity (maximal number of successive differentiations carried out)	5.0	6.7	5.0	7.1
127—115	500—900	Number of coincidences before the development of a stable differentiation	4.3	4.8	2.5	3.0
		Stability of the differentiations effected (in %)	65.4	82.9	71.0	84.5
		Regularity (maximal number of successive differentiations carried out)	3.8	5.0	3.8	7.1
127—115	500—800	Number of coincidences before the development of a stable differentiation	2.7	6.2	2.2	2.5
		Stability of the differentiations effected (in %)	79.6	81.2	84.1	89.1
		Regularity (maximal number of successive differentiations carried out)	4.5	6.4	5.3	7.0
127—117	500—700	Number of coincidences before the development of a stable differentiation	2.1	2.8	1.2	2.5
		Stability of the differentiations effected (in %)	74.1	83.5	81.9	81.6
		Regularity (maximal number of successive differentiations carried out)	6.9	5.9	6.7	7.3

TABLE 3

Figures Showing Formation of Conditioned Reflexes in Control and Experimental Chicks with Portions of the Hemispheres Removed

Indication of formation of conditioned reflexes (differentiations)	Kind of conditional reflexes			
	visual		auditory	
	experiment	control	experiment	control
Number of coincidences required before the first appearance of a conditioned reflex	—	3.9	1.3	1.5
Number of coincidences before stabilization of reflex	4.9	8.2	1.3	2.3
Stability of the conditioned reflex (positive responses as % of the total)	97.9	80.6	96	86.4
Regularity of the conditioned reflex (maximum number of successive positive responses)	19.2	17.0	16.9	15.4
Latent period of the conditioned reflex (in seconds)	1.9	3.0	2.0	2.8

Initially, for 2 days, all the chicks were taught to open the troughs and to obtain food from them in the illuminated compartment. After this an association was developed between light stimulation and feeding. After forming and consolidating the conditioned reflex until 10 successive positive reactions were obtained, we then proceeded to differentiate the reaction to changes in strength of the light signal (which was given at voltages of 60, 80, 90, 100, 110, 115, and 117 volts). Differentiation of the sound stimuli was made in terms of pitch, using frequencies of 3000, 2000, 1500, 1000, 900, 800, and 700 c/s. Thus, 7 light and 7 sound differentiations were made, beginning with the coarsest and ending with the finest.

At the end of the experiments in which the conditioned reflexes and their differentiation were established, the animals were killed and the brains examined.

RESULTS

Table 1 shows the average times for the formation of the conditioned reflexes, their stability, and consistency, as measured in experiments on the 10 control chicks and in all the operated animals in which the whole of the posterior half of the hemispheres was removed at an early age.

It can be seen from Table 1 that the number of coincidences required for the first manifestation of a conditioned reflex are quite different in the case of the visual and auditory stimuli. In all other tests, the experimental animals differ from the controls; they differ in the required reinforcement of the conditioned reflexes, their stability, regularity, and also in the latent period of the motor reactions. Thus, removal of the posterior halves of the cerebral hemispheres in chicks, carried out so as to include the cortex and part of the striatum, leads to some disturbance in the ability of the brains to form new pathways, a disability which is not compensated or restored as the animal develops.

Figures showing the development of differentiations, and their stability and consistency, are shown in Table 2.

It can be seen from Table 2 that in developing differentiations, apart from the first, the rate in the operated animals is not only not lower than in the controls, but may even be higher, though the reflex is less stable and less regular.

Thus, removal of brain substance including portions of the corpora striata causes a marked interference with the action of the visual and auditory stimuli. There is no recovery from this perceptual disturbance, even after long training.

TABLE 4

Figures Showing the Development of Differentiation in Animals with Portions of the Hemispheres Removed, as Compared with Controls

Differentiated stimuli		Quantities indicating formation of conditioned reflexes (differentiations)	Kind of conditioned reflexes (differentiations)			
			visual		auditory	
light (direct voltage applied to lamp)	Sound (tone in cps)		experiment	control	experiment	control
127—60	500—3000	Number of coincidences before the development of a stable differentiation	4.1	16.9	3.6	5.4
		Stability of the differentiations effected (in %)	78.8	76.8	83.2	83.3
		Regularity (maximal number of successive differentiations carried out)	5.0	8.5	4.9	10.9
127—80	500—2000	Number of coincidences before the development of a stable differentiation	2.4	6.6	3.4	2.4
		Stability of the differentiations effected (in %)	86.6	85.0	87.6	86.0
		Regularity (maximal number of successive diffferentiations carried out)	5.6	7.9	5.5	6.8
127—90	500—1500	Number of coincidences before the development of a stable differentiation	1.9	7.8	2.8	2.3
		Stability of the differentiations effected (in %)	90.0	84.1	87.3	81.9
		Regularity (maximal number of successive differentiations carried out)	5.1	6.5	5.0	7.5
127—100	500—1000	Number of coincidences before the development of a stable differentiation	4.8	8.0	3.1	3.1
		Stability of the differentiations effected (in %)	78.5	81.1	84.0	82.3
		Regularity (maximal number of successive differentiations carried out)	4.7	6.7	4.1	7.1
127—110	500—900	Number of coincidences before the development of a stable differentiation	4.1	4.8	2.9	3.0
		Stability of the differentiations effected (in %)	80.1	82.9	86.6	84.5
		Regularity (maximal number of successive differentiations carried out)	5.4	5.0	5.5	7.1

Table 3 shows the results of the average values of the formation, stability, and regularity of the conditioned reflexes developed in chickens in which the cortical rudiments were removed at an early age.

As can be seen from Table 3, the operated chicks perform every bit as well as the controls, and may even surpass them.

Table 4 shows the results of experiments in which a differentiation was made between light stimuli and between sound stimuli. At first large differences were used (127 and 80 volts applied to the lamp, and tones of 500 and 3000 c/s), after which the differentiation was refined.

TABLE 5

Measurement of Conditioned Reflex Activity in Chickens in Whom the Posterior Halves of the Hemispheres had been Removed when Fully Grown

Indication of formation of conditioned reflexes (differentiations)	Kind of conditioned reflexes			
	visual		auditory	
	before operation	after operation	before operation	after operation
Stability of the conditioned reflexes (as % of positive responses)	89.1	85.7	94.4	100
	75.4	77.7	94.6	77.7
	81.1	100	93.6	100
Stability of the differentiations (as % of total number of tests)	100	87.5	97.5	100
	100	79.1	98.0	66.6
	83.3	33.3	85.0	16.6
Latent periods of the conditioned reflexes (in seconds)	2.5	1.9	2.8	2.6
	6.4	3.2	3.8	3.4
	5.0	2.7	3.9	5.3

TABLE 6

Change in Conditioned Reflex Activity of Chickens in Whom the Cortical Structures had been Removed in the Adults

Indication of formation of conditioned reflexes (differentiations)	Kind of conditioned reflexes			
	visual		auditory	
	before operation	after operation	before operation	after operation
Stability of the conditioned reflexes (as % of positive responses)	83.5	54.0	69.0	47.2
	89.3	100	94.1	100
Stability of the differentiations (as % of total tests)	100	100	93.7	100
	100	100	68.7	100
Latent periods of conditioned reflexes (in seconds)	3.0	5.0	4.0	2.8
	1.7	2.2	1.3	2.7

It can be seen that the operated animals perform as well as the controls, and in some cases even better. Thus, removal at an early age of the superficial portions of the hemispheres with their cortical rudiments causes no noticeable disturbance of conditioned reflex activity, such as occurs in the 8-12 month animals.

Removal of Portions of the Hemispheres in Adult Chickens

The experiments on these birds were arranged so that at first conditioned reflexes and differentiations were elaborated, after which portions of the hemispheres were extirpated. After recovering from the operation, tests were made of the state of the positive and differentiated conditioned reflexes.

In 3 adult birds, the posterior halves of the hemispheres including portions of the corpora striata had been removed. The results of the conditioned reflex study are shown in Table 5.

From Table 5 it can be seen that after the operation not only was there no reduction in the stability of the conditioned reflexes already elaborated, but that in most cases they became even more stable when the reflex was reinforced; there was a noticeable reduction in the latent period of the reactions. However, removal of the posterior portions of the hemispheres, including the corpora striata, caused a profound disturbance of the negative conditioned reflexes. This was shown by a marked reduction in the stability of previously elaborated differentiations.

The superficial layers of the hemispheres including the cortical rudiments were removed from 2 adult birds in whom conditioned reflexes and differentiations had been elaborated. The results obtained in these experiments are shown in Table 6, from which it can be seen that removal of the cortex caused a deterioration in one case and an improvement in the other of the stability of the positive conditioned reflexes. In most cases the latent periods were increased. However, the stability of the differentiations was not only not reduced by the operation, but, as can be seen from the example of the differentiated response to the auditory stimuli, it was actually increased. It is not possible to draw any further conclusions from the results on this group of adult operated birds, because of the scatter in the results and the small number of animals concerned.

As a result of these observations it was established that conditioned reflex activity was maintained in chickens in which the occipital portions of the cortex were removed at an early age, and that perceptual function was damaged in birds in which, in addition, there was damage to the corpora striata. This result confirms the importance of the corpora striata in forming circuits in a brain in which the cortex is phylogenetically immature [4, 6, 7]. It is not unexpected that the disturbance of the conditioned reflex activity is such that responses to positively conditioned stimuli are as a rule preserved, but there is a reduced ability to differentiate between stimuli. This may indicate that simple temporary connections may be formed in the portions of the corpora striata remaining, and that the whole striatal system (or, in any case, of its posterior portions) may participate in the more complex analytical acts.

Morphological studies of the brain of the operated animals showed that extirpation at an early age which includes the corpora striata in most cases causes irreversible damage, but that when only part of the occipital cortex is removed, no visible damage can be discerned after 6-7 months.

It has been shown that in mammals, regeneration of portions of the cerebral hemispheres removed at an early age may take place [3, 5, 8]; this occurs only in birds for the cortical region, and does not apply to the corpora striata which form the main mass of the cerebral hemispheres.

SUMMARY

Removal of the cerebral hemispheres, including the corpora striata, in young chicks has a marked effect on their subsequent conditioned reflex activity. The stability and regularity of the conditioned reflexes is reduced, and differentiation of the visual and of the auditory conditioned signals deteriorates. Removal of the rudimentary cortical areas in young chicks has little effect on their subsequent reflex activity, and does not affect accurate visual or auditory differentiation. Disturbances of visual and auditory analysis caused by injury to the corpora striata are found also in adult hens, but do not occur when only the rudimentary cortical areas are destroyed. Cortical areas removed from young chicks regenerate, while there is no recovery from damage to the corpora striata.

LITERATURE CITED

[1] B. I. Bayandurov, The Trophic Function of the Brain•. Medgiz, 1949.

[2] I. S. Beritov, Pflugers Arch., 1926, Bd. 213, S.370.

[3] N. N. Dzidzishvili, in book: Reports of the 2nd Trans-Caucasian Congress of Physiology, Biochemistry, and Pharmacology•. Izd. AN Georgian SSR. Tbilisi, 1956.

[4] A. I. Karamyan, The Evolutionary Function of the Cerebellum and Cerebral Hemispheres•. Moscow, 1956.

[5] A. B. Kogan and T. V. Ivannikova, Byull. éksptl. biol. i med., volume 39, No. 3, pp. 6-9 (1955).

•Original Russian pagination. See C. B. Translation.

[6] L. A. Milyutina, in book: Research Reports of the 2nd Moscow State Medical Institute* ,Moscow, 1958, p. 69.

[7] V. V. Petelina, in book: Problems of the Comparative Physiology of Higher Nervous Activity* , edited by D. Biryukov, Institute of Evolutionary Morphology, AMN SSSR, Leningrad, 1958.

[8] A. V. Polezhaev, A. I. Matveeva and N. A. Zakharova, Reports of the AN SSSR,* volume 113, p. 472 (1957).

[9] M. Schrader, Pflugers Arch., 1889, Bd. 240, S. 503.

[10] P. Flourens, Recherches expér. sur les fonctions du systeme nerveux. Paris, 1842.

* In Russian.

PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

RESTORATION OF VITAL FUNCTION IN MONKEYS AFTER BLEEDING TO DEATH DURING HYPOTHERMIA

V. A. Negovskii, V. I. Soboleva, N. L. Gurvich,

K. S. Kiseleva and Sh. S. Machavariani

From the Laboratory of the Experimental Physiology of Resuscitation (Head – Professor V. A. Negovskii) AMN SSSR, Moscow, and of the Institute of Experimental Pathology and Therapy (Director – Doctor of Biological Sciences I. A. Utkin), Sukhumi

(Received February 20, 1959. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

It has been shown recently both in clinical and laboratory work that the central nervous system is considerably more resistant to temporary interruption of the circulation when the temperature is artificially reduced. Previous laboratory investigations have shown that artificial cooling enables the period of "clinical death" in dogs caused by exsanguination to be increased from the normal value of 5-6 minutes up to one hour, and that afterwards there may be a complete recovery [2].

We have carried out similar experiments on monkeys as representing animals at higher stages in evolution, so that the results might be more closely applicable to clinical practice. There are not many reports dealing with hypothermia in monkeys, but it has been emphasized [4, 7, 8, 9] that in such experiments certain specific features of monkeys distinguish them from other animal species.

METHOD

The experiments were carried out on 10 monkeys of both sexes, chiefly hamadryads, age from 3 to 14 years, and weighing from 6 to 26 kg.

Injections of 1.5 ml per kg of a 2% solution of pantopon, and 0.1 mg per kg of a 0.1% atropine solution were given before the experiment. The temperature was reduced by surrounding the animal with ice packs.

Before and during the cooling, an intravenous drip of 0.2% pentothal solution was given. Clinical death during the hypothermia was caused by bleeding from the femoral artery.

Restoration of vital function was brought about by a complex method which consisted of forcing the arterial blood in towards the center, applying artificial respiration, and in many cases by massaging the heart directly. When the heart began to fibrillate, a single electrical impulse was applied to it. Immediately the heart began to beat; in order to combat the acidosis, an intravenous injection of 0.15 g per kg of sodium bicarbonate in an 8% solution was given. In most of the experiments the body temperature was raised by immersing the animal in water at 43-45°.

RESULTS

As has already been pointed out, the animal was cooled during pentothal anesthesia. The total amount of pentothal injected varied from 70 to 150 ml. In spite of the fact that two to three times less pentothal was given to monkeys than to dogs in similar experiments, it was found that the monkeys were exceptionally sensitive to

this anesthetic. In 5 of the 10 animals, the respiration became irregular during the cooling, particularly during the first hour, Cheyne-Stokes breathing occurred, and sometimes even this kind of breathing ceased. It was therefore neccessary to apply artificial respiration during cooling. On the other hand, if the pentothal injection was discontinued for a short period, the animal recovered from the anesthetic and an active reaction to the cold took place. An increase of approximately 10 times in the toxicity of anesthetics in the cold has been repeated [5]. In the remaining 5 monkeys, nothing but a slight slowing of the respiration rate was observed.

In 2 of the 10 monkeys, after 1 hour, 48 minutes and after 2 hours, 2 minutes from the beginning of the cooling, when the body temperature had fallen to 27.3°, extra-systoles appeared, and these indicated the onset of fibrillation. In 1 monkey the fibrillation was rapidly eliminated, while in the other, repeated attempts to do so were successful only for a short time, after which it again reappeared. Fibrillation continued for 25 minutes. During this time respiration ceased. Subsequently, elimination of the fibrillation, warming the animal, intravenous injection of warm polyglucin solution, and artificial respiration restored the heartbeat and respiration. After 2 hours from the onset of cooling, the monkey was once more in good condition. Thus, unlike other animals, the monkeys tolerated clinical death occurring as a result of cardiac fibrillation even when this developed during the cooling process.

In the remaining 9 animals, 1 hour, 13 minutes and 2 hours, 30 minutes from the onset of cooling when the temperature had fallen to 26.2-28.5°, bleeding was continued. The arterial pressure fell from 120-150 to 72-100 mm mercury. The highest pulse rate before the experiment was 150-200 per minute, and during bleeding it fell to 58-100 per minute. The duration of the ventricular complex increased from 0.2 to 0.35 second. Changes in the electrocardiogram consisted of a marked increase in the amplitude of the negative going wave, in an expansion and division into two of the R notch and the appearance of an extra spike on the initial portion of the S-T wave ("injury potential"). It must be noted that this change was observed in dogs only when the temperature was cooled below 25°. The respiration rate varied from 11 to 22 per minute.

In 8 of the 9 animals, the time taken to die varied from 42 minutes to 2 hours, 16 minutes, i.e., it was considerably longer then in the corresponding experiments on dogs. Only in one monkey was the period shorter, lasting 22 minutes, 10 seconds. In one animal respiration ceased during cooling but before bleeding, and for the first 11 minutes of the period for which the animal was dying, artificial respiration was maintained before natural breathing was restored.

The long time which the animals took to die in some of the experiments must be explained as being due to very highly developed compensation. Thus, for instance, during the bleeding, and particularly when it was first begun, there was frequently a spasm of the peripheral vessels. The amount of blood which was obtained by free bleeding was not more than 30-50% of the total amount. In 6 out of the 9 animals, after regular respiration had ceased, a period of agony ensued, and lasted for 11-20 minutes. Despite the fact that long before the agony, at the 8-15th minute of the dying period, the arterial pressure fell to zero, respiration was maintained for as long as from 25 to 42 minutes, and in one experiment continued for as long as 2 hours after the fall in arterial pressure. The long-maintained functioning of the respiratory center in monkeys at blood pressure levels approximating to zero must be regarded as a special form of compensation which is far more highly perfected than in dogs, where such an event never occurred. The early spasm of the peripheral vessels, by reducing blood loss, maintains an adequate oxygen supply for a considerable period [3] and so protects the nervous centers. Support for this view is supplied by clinical observations in which consciousness is maintained in dying individuals in whom no blood pressure can be recorded.

Before bleeding there was little change in the ECG. As the hypoxia increased, the P wave became broader and there was a displacement of the S-T interval upwards, so that the ventricular complex showed up as a monophasic deviation. During the terminal pause and cessation of sinus control, occasional biphasic complexes occurred in abnormal positions.

Clinical death lasted from 10 to 30 minutes. At the end of this period the body temperature was 21-27.2°. In one monkey it was below 17°. During this time occasional monophasic excursions of the line corresponding to the ventricular complex were observed on the ECG. Usually these disappeared completely by the 5-10th minute, and if no fibrillar oscillations occurred, there was no further ECG activity until the blood was forced into the arteries.

The heartbeat was restored in from 27 seconds to 2 minutes from the beginning of resuscitation. As the blood was driven into the arteries, ventricular complexes began to appear on the ECG, and the changes observed

were the reverse of those recorded while the animal was dying. The return of the ECG to its original level occurred when the body temperature was raised to 32-34°.

With the exception of two animals, after the heartbeat had been restored, there was fibrillation. In many of the monkeys it was rapidly eliminated by applying a single electrical impulse to the heart. In 4 animals it returned many times during the first 22-34 minutes of recovery. Spontaneous arrest of the fibrillation occurred frequently, both while the animal was dying, during clinical death, and during recovery, though this never happened in dogs. Subsequently, however, fibrillation again returned, when it was once more eliminated electrically. Subsequent arterial injection of warm blood with added glucose and hydrogen peroxide led to permanent restoration of normal heart function. Cases have previously been reported of spontaneous cessation of fibrillation in monkeys [6].

In one experiment, an ineffectual heartbeat was restored by massaging the heart and driving the blood into the arteries 1 hour, 29 minutes after the onset of recovery. During the whole of this time the heart fibrillated, evidently due to the low temperature of 17°.

After the restoration of heart function, the weak beat had to be maintained by massage. Respiration was restored 2 hours, 15 minutes from the beginning of cooling, and then again failed.

In the remaining animals the respiration was restored at times from 3 minutes, 15 seconds to 24 minutes after the beginning of recovery, when the body temperature was 23-27°. In 4 monkeys it was restored, but the heart fibrillated. Short-term restoration of the beat in the fibrillating heart which sent a periodic supply of blood to the brain, and artificial respiration restored normal breathing before the normal heartbeat returned. In one experiment the respiration was never re-established. The respiration returned to normal rapidly (after 60-90 minutes), and such an effect is now found in dogs. It may be to some extent associated with the action of the bicarbonate. Several descriptions have been given [1] of the rapid restoration of breathing after injecting bicarbonate into animals who have survived 5 or 7 minute periods of clinical death at normal temperatures.

In monkeys, the pupil reflexes were restored 24 to 57 minutes after the beginning of recovery when the body temperature had values between 24.1 and 30°.

In 5 of the 9 animals, the recovery of the heartbeat and the respiration was only temporary. After recovering, the animals died from pulmonary edema, 2 dying on the operating table and one at the end of the first day. Drawing off the fluid, bleeding, injecting of calcium chloride and 40% glucose, did not prevent the development of edema. In another animal, death occurred during heart massage when the artificial respiration was wrongly applied and pulmonary tissue was torn, the need for a right pneumothorax being realized too late. It must, however, be appreciated that two of the monkeys which died were of the Macaque-Lapunder breed. These animals are extraordinarily sensitive to operative measures, they do not become acclimatized or breed in the conditions obtaining in Sukhumi.

Complete restoration of vital function in animals which have survived clinical death from bleeding during hypothermia occurred in 4 of the 9 monkeys. Two of these survived a 10 minute period of clinical death, 1 a 20 minute period, and in 1 the condition lasted for 30 minutes. It is important to note how rapidly vital function was restored in these cases. Hearing and sight returned after 4-10 hours from the beginning of cooling. After 10-18 hours, they were able to stand unaided. For a short time there was some discoordination of movements. Complete and permanent recovery occurred after 19-48 hours. Recovery was particularly rapid in the animal which had been in a condition of clinical death for 30 minutes. After 6 hours, 30 minutes from the beginning of recovery, its outward appearance was almost normal.

Thus, by means of hypothermia it was possible to extend clinical death from bleeding for as long as 20-30 minutes.

It must be noted, however, that unlike dogs, even under hypothermic conditions, monkeys were more sensitive to severe hypoxia caused by bleeding to death. Recovery in these cases represents a more complex process. Thus, for instance, of the 4 monkeys which survived 30 minute periods of clinical death, only one recovered completely.

At the same time it is known that hypothermic dogs which have survived such periods as a rule recover permanently [2].

Also, when monkeys recover, vital function returns considerably more rapidly than in dogs, and this is evidently to be ascribed to their more highly perfected compensatory processes.

SUMMARY

Clinical death resulting from acute blood loss in monkeys was prolonged up to 20-30 minutes with the aid of hypothermia. Vital function was restored by means of centripetal arterial blood transfusion and artificial respiration; direct cardiac massage and elimination of defibrillation by electrical stimulation were used in a number of cases. Monkeys are, however, more sensitive to the hypoxia caused by bleeding to death than are dogs, even under hypothermic conditions. Thus, complete restoration of vital function was achieved only in 1 monkey out of the 4 which sustained a 30 minute period of clinical death. At the same time, when the monkeys recover, vital function is restored more rapidly than in dogs. The latter is evidently due to more perfect compensation.

LITERATURE CITED

[1] O. N. Bulanova, Bulletin of the AMN SSSR, No. 2, p.79 (1957).

[2] V. A. Negovskii and V. L. Soboleva, Arkh. pat., No. 6, p.58 (1956).

[3] A. Labori and P. Gyugenar, Hibernation Therapy (Artificial Winter Sleep) in Medical Practice*, Moscow, 1956, pp. 83-85.

[4] W. G. Bigelow and I. E. McBirnie, Ann. Surg., 1953, v.137, p.361.

[5] A. M. Dogliotti and E. Ciocatto, Lyon chir., 1954, v.49, p.19.

[6] P. Goffrini and E. Bezzi, Lipothermia generate controllata in chirurgia, Pisa, 1954.

[7] J. Malmejac, P. Plane and E. Bogaert, Compt. rend. Acad. sc., 1956, v.242, p.2171.

[8] J. D. McMurrey, W. F. Bernhard and J. A. Taren, Surg. Gynec. and Obst., 1956, v.102, p.75.

[9] S. A. Niari and F. J. Lewis, J. Appl. Phys. 1957, v.101, p. 137-138.

* In Russian.

COMPENSATORY CHANGES IN THE KIDNEY AFTER UNILATERAL NEPHRECTOMY

V. I. Fedenkov

Course of Pathological Physiology and Laboratory Diagnosis (Head – Docent M. L. Kolpakov) Stanlin Institute of Higher Medical Study (Director – Docent G. L. Starkov)

(Received January 12, 1959. Presented by Active Member of the AMN SSSR V. V. Parin)

Compensatory processes are among the more important of the adaptive mechanisms. Defects due to damage of one of a pair of organs may be largely made good by compensation on the part of the uninjured member. Such a compensatory process may be involved in the recovery of human patients.

Simon in 1867 investigated compensation following unilateral nephrectomy. He showed that after removal or functional elimination of one kidney, there is a considerable increase in the activity of the other. This process included firstly the involvement of reserve nephrons and an increase in blood flow, and there was also a hyperplasia and hypertrophy of the nephron cells [6, 7, 8].

A great deal of work has been done on compensatory changes following unilateral nephrectomy. However, these were carried out at a time when methods had not yet been developed which allowed detailed renal investigations to be made [5, 9].

At present, owing to the development of more perfect methods of study of renal function [3, 11, 12, 13], it has been possible to make a full investigation of functional changes involved in the compensatory reorganization of the remaining organ, and such methods have been used in the present study.

METHOD

The experiments were carried out on 6 female dogs aged 3 to 4 years in which an operation using the method of Pavlov-Orbeli was performed. The filtration rate was measured in terms of thiosulfate clearance (F) and was calculated from the formula $F = \frac{ID}{P}$, where P represented the thiosulfate content in mg per ml of plasma; I the thiosulfate content in mg per ml of urine; D the diuresis in ml per minute. The reabsorption of water (R) was determined from the formula R of water = F-D, where F is the filtration rate in ml per minute.

For an accurate estimation of the filtration and reabsorption rates, it is necessary to maintain the concentration of the substances concerned at a constant level. This was achieved by using an intravenous drip. At the start of the experiment, an amount of water equal to 5% of the body weight was introduced into the stomach through a tube. For 10 minutes a solution containing 3.5% sodium thiosulfate was introduced as a drip. The urine for analysis was collected 25 and 40 minutes after giving the water. Blood was taken halfway between these two times. The concentration of sodium thiosulfate in the urine and in the plasma was determined by titrating with iodine.

The results were expressed as ml per minute and referred to unit surface area. This method made it possible to compare the results obtained on various animals [3]. In order to obtain a complete picture of the compensatory functional changes in the remaining kidney, all the results of the experiment on each dog were averaged over a period of one month. This procedure is necessary on account of the considerable fluctuations in renal function.

TABLE 1

Changes in the Diuresis of the Right Kidney after Removal of the Left (in ml per minute per m^2)

Name of dog	Before operation	After operation, monthly averages			
		I	II	III	IV
Swallow	6.1	10.9	17.9	18.7	14.7
Naida	6.6	16.2	13.6	11.3	10.3
Rose	7.6	10.1	9.4	19.3	7.1
Lady	6.9	10.6	15.5	17.9	15.7
Palm	7.6	9.1	8.3	8.3	7.9
Gerta	8.0	11.3	12.0	13.0	12.5

TABLE 2

Changes in the Filtration Rate of the Right Kidney after Removal of the Left (in ml per minute per m^2)

Name of dog	Before operation	After operation, monthly averages			
		I	II	III	IV
Swallow	74.3	91.1	119	140	99
Naida	80.2	90.1	97.6	92.5	85.8
Rose	62.0	65.7	83.1	77.3	67.2
Lady	74.4	98.6	147	144	121
Palm	47.8	65.8	62.2	66.8	58.3
Gerta	65.5	94.3	70.7	86.3	78.3

After carrying out 5-6 determinations to establish the normal renal function, the left kidney was removed. Measurements on the function of the remaining organ were made daily for four months.

RESULTS

The results showing rates of diuresis, filtration, and water reabsorption by the remaining kidney are shown in Tables 1, 2, and 3.

As can be seen from the results given in the tables, the diuresis after one month is far greater than the original values (on the average, by 140%).

Subsequently, in 3 of the 6 animals, the diuresis increased still further. During the fourth month the rate in these animals was somewhat reduced, and approximated to the values found during the first month following the operation.

For 3 dogs (Naida, Rose, and Palm) the maximum increase in the diuresis occurred in the first month after the operation, after which it returned almost to its original level.

One month after the operation there was an increased filtration in all cases. Subsequently, in most of the animals, there was a further increase, and in some the maximum occurred in the second month after the operation, and again in others in the third. In Gerta, the greatest filtration rate was attained one month after the operation, after which it fell somewhat. In the fourth month the increased filtration showed some reduction, and in all the animals it returned to the value found during the first month after the operation.

The absolute values of the monthly averages of the water reabsorption increased, just as did the diuresis and filtration. Water reabsorption in the third month increased in all the animals to 140% of the original value.

It was found that the function of the remaining kidney underwent considerable change. These changes are expressed as the absolute values of diuresis, filtration, and water reabsorption. It must be appreciated that all three quantities change in the same way.

TABLE 3

Changes in the Water Reabsorption Rate of the Right Kidney after Removal of the Left

Name of dog	Absolute values in ml per minute per m^2					Reabsorption, as percentage of filtration				
	before operation	after operation, monthly averages				before operation	after operation, monthly averages			
		I	II	III	IV		I	II	III	IV
Swallow	68.2	80.2	101.1	121.3	84.3	91.8	88	84.9	86.6	84.1
Naida	73.6	73.9	84.0	81.2	75.5	91.7	82	86.1	87.7	88
Rose	54.4	55.6	73.7	68.0	60.1	87.7	84.6	88.7	88	89.4
Lady	67.5	88	131.5	126.1	105.3	90.7	89.2	89.4	87.6	87
Palm	40.2	56.7	53.9	58.5	50.4	82.2	86.2	86.6	87.6	86.4
Gerta	57.5	83	58.7	73.3	65.8	87.8	88	83	84.9	84

The determination of the reabsorption as a percentage of the filtration is a considerable help in interpreting the results.

When the water reabsorption is expressed in this way, it can be seen that in spite of the increased absolute amount of reabsorption in the remaining kidney, the percentage reabsorption was in all cases reduced below the original value. Calculation of the percentage water reabsorption reveals the reason for a certain difference in changes in the diuretic rates. Thus, for example, the maximum increase in Naida in the first month after operation can be seen to be due not to increased filtration, but to a marked reduction in water reabsorption; this effect cannot be seen from the absolute figures, but is apparent immediately when water reabsorption is expressed as a percentage of filtration (82% as compared with 91.7% before the operation).

In Palm, 2-3 months after the operation the filtration increased considerably, but the small increase in diuresis at this time was probably due to an increased water reabsorption (87.6% as aganist 82.2% before the operation).

Regulation of renal function is complex and consist of many reflex processes including hormonal action [1, 4].

Probably changes in the rates of diuresis, filtration, and reabsorption are evidence of compensatory reorganization of the remaining kidney which are brought about by changes in neuro-humoral regulation.

SUMMARY

Compensatory changes in the intact kidney following unilateral nephrectomy in dogs were studied. Diuresis, filtration, and water reabsorption were determined. The absolute values of diuresis, filtration, and water reabsorption were increased in the majority of animals after 2-3 months from the time of the operation.

In the author's opinion, the compensatory changes in the intact kidney is due to alterations in neuro-humoral regulation.

LITERATURE CITED

[1] K. M. Bykov, The Cerebral Cortex and the Internal Organs*. Moscow-Leningrad, 1947.

[2] G. F. Blagman, Z. N. Estrin and E. B. Dvorkina and others. Klin. med., volume 29, No.5, p.59 (1951).

[3] V. F. Vasil'eva, Fiziolog. zhurn., volume 44, No. 3, p.236 (1958).

[4] A. G. Ginetsinskii, Course of Normal Physiology; Textbook for Medical Institute*. Moscow, 1956.

[5] A. G. Martynyuk, The Condition and Function of a Kidney Remaining After Nephrectomy*. Author's abstract of dissertation. Stanislav, 1949.

*In Russian.

[6] F. Z. Meerson, The Mechanism of Compensatory Renal Hypertrophy and Its Stimulation by Physical Agents*. Candidate's dissertation. Yalta, 1951.

[7] V. V. Podvysotskii, Foundations of General and Experimental Pathology*. St. Petersburg, 1905.

[8] P. P. Yur'ev, in book: Medical Supplement to Sailor's Compendium*, 1899, September, p.152; October, p. 231.

[9] L. Ambard, Presse méd., 1926, v. 100, p. 1569.

[10] P. B. Rehberg, Bioch. J., 1926, v. 20, p.447.

[11] J. A. Shannon, J. Clin. Invest., 1935, v.14, p. 403.

[12] J. A. Shannon, Am. J. Physiol., 1936, v. 117, p. 206.

* In Russian.

CENTRAL NERVOUS CHANGES IN EXPERIMENTAL TETANUS AND THE MODE OF ACTION OF THE TETANUS TOXIN

COMMUNICATION II. THE PART PLAYED BY SPINAL MECHANISMS

G. N. Kryzhanovskii

From the Laboratory of the Pathology of Infection (Head – Corresponding Member of the AMN SSSR Professor A. Ya. Alymov) Section of General Pathology (Head – Academician A. D. Speranskii) Institute of Normal and Pathological Physiology (Director – Active Member of the AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Received May 22, 1959. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

In previous articles [5, 6] we have described the characteristic irradiation of excitation in the central nervous system of animals infected with general ascending tetanus. The characteristic feature of this phenomenon in the development and generalization of excitation in the central nervous system is facilitated by applying various stimuli to the limb into which the tetanus toxin was injected; (in future, for convenience this will be referred to as the "tetanized" limb). When similar stimuli are applied to other parts of the body, including the opposite limb, which was also involved in the pathological process (segmental tetanus) the spread of the excitation along the central nervous system was either poorly shown, or did not occur at all. The investigations showed [5, 6] that the phenomenon cannot be due to any peripheral mechanism, such as, for instance, an increased excitability of the receptors of the "tetanized" limb, which would cause additional central nervous stimulation.

The present work concerns a further analysis of the phenomenon described. The problem is to explain the part played by the facilitatory mechanisms described above in the development of spasms induced by stimulating the tetanized extremity, and to investigate the involvement of other parts of the nervous system, in particular the dorsal root ganglia. Solution of these problems is of interest from the neurological standpoint, as it concerns the spastic syndrome characteristic of tetanus.

METHOD

The experiments were carried out on white rats weighing 140-160 g. In order to eliminate suprasegmental influence, we divided the spinal cord [21] between C_4 and C_5. Under these conditions, natural breathing is maintained. To eliminate the influence of the spinal ganglia conveying afferent stimuli to the cord, the normal stimulation from the receptors was replaced by electrical stimulation of the central ends of the cut dorsal roots. The dorsal roots of L_4 to S_1 were cut, and in several experiments those of L_3 and S_2 were also divided, thus interrupting the sensory inflow from muscles innervated by the sciatic nerve. Stimulation was applied to L_5 or L_6. Section of the spinal cord and dorsal roots was made under a brief ether anesthesia; during the experiment, careful experiments were made on the excitability of the spinal cord, and care was taken to avoid cooling or damaging the animal unnecessarily; in many experiments, the spinal cord was surrounded with vaseline.

The experiments were carried out during the development of ascending generalized tetanus several days after injecting 3 times the minimum lethal dose (MLD) into the muscle of the left hind limb which is innervated by the sciatic nerve (gastrocnemius and posterior thigh muscles). Simultaneously with the toxin, 0.025 active units (AE) of serum were injected. Other details of the method have been described in detail in [5].

RESULTS

Tests have shown that section of the spinal cord has no appreciable effect on the appearance of the phenomena described: just as before section, application of various stimuli to the tetanized limb (pressing on the muscle, tendon, or foot; bending the paw; pinching the skin of the pad of the foot, etc) induced the characteristic onset of generalized tetanic convulsions embracing all muscles of the trunk and limbs lying distal to the point at which the cord was sectioned. During this time, in the muscles referred to, a powerful volley of electrical impulses was recorded. The upper two traces in the drawing show the electrical activity in the muscles of the right thigh during a convulsion induced by pressing the paw of the tetanized limb. Similar pressure on the opposite (right) paw caused only a local response consisting of a withdrawal of the foot and the appearance in the muscles of a short-lasting and comparatively small burst of electrical activity (see lowermost trace of figure).

Thus, after elimination of the higher nervous centers, stimulating the tetanized limb causes a generalized excitation throughout the whole spinal cord involving remotely placed motor neurons. A particular feature of this reaction is the long after-effect and the slow extinction of the electrical activity, with separate repeated afterbursts (see Figure, A). The latter occur particularly frequently in the muscles of the opposite hind limb, but also may frequently be recorded simultaneously in different muscles of the trunk and limbs, thus indicating a snychronization of the bursts of excitation of the motor neurons in different segments of the spinal cord.

These features of the reaction, i.e. , the low threshold, high intensity, and involvement of different muscle groups including antagonist muscles, the long after-effect, the slow extinction, the appearance of repeated "spontaneous" discharges of motor neurons, and the synchronization of the bursts of their excitation, represent features characteristic of spasticity [17, 18]. G. Magoun and other workers [18, 21, 22] have shown that an important part in the development and maintenance of this condition is played by the supraspinal structures, in particular by the reticular formation of the brainstem. Our results show that this phenomenon may be brough about by the intraspinal mechanisms without the participation of the suprasegmental facilitatory influences. It does not follow, of course, that supraspinal mechanisms play no part in tetanic convulsions. This problem will be investigated separately.

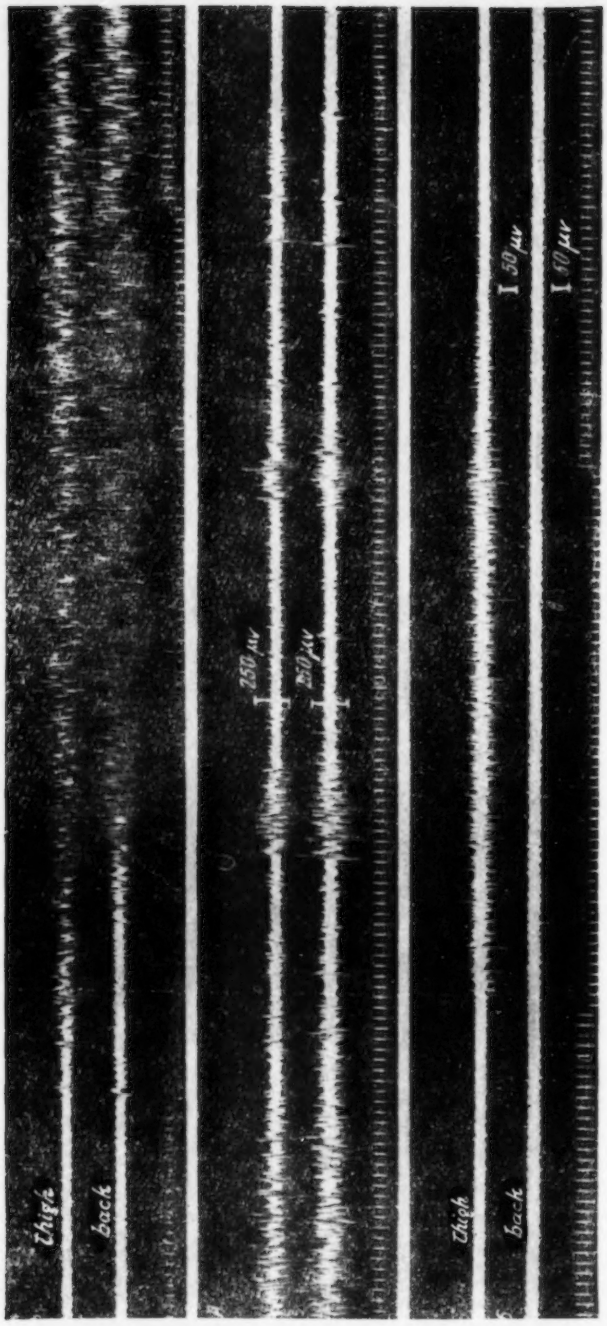
Experiments in which the central ends of the cut dorsal roots were stimulated while the cord was intact showed that stimulation of L_5 or L_6 on the left side, which was the side in which the toxin injection was given, caused a typical attack of tetanic convulsions with a high level of electrical activity in the different muscles of the trunk, neck, and limbs; there was a long after-effect with repeated bursts of impulses. The tetanic reaction could be induced even with very mild stimulation, it always became generalized, and when the tetanus infection was severe it always appeared immediately at maximum strength. When a similar stimulus was applied to the central ends of the cut roots of the opposite side, there was only a small response, and in order to invoke a generalized spasm, the strength of the stimulus had to be increased considerably. However, in the latter case the convulsion was much weaker than when the roots on the tetanized side were stimulated.

The experiments showed, therefore, that even with direct afferent stimulation of the spinal cord without any possible participation of the spinal ganglia, the development of a generalized spasm is facilitated, i.e. , there is a facilitation of the spread of excitation along the central nervous system when the stimulus is applied on the tetanized side. Owing to section of the dorsal root, any positive feedback from muscles innervated by the sciatic nerve and into which the tetanus toxin had been injected was eliminated; the phenomenon cannot therefore be due to any additional proprioceptive stimulation corresponding to a servo-reflex based on feedback.

The results obtained agree entirely with previous investigations [5, 6] and indicate that the phenomenon is due to changes in the functional condition of the central reflex apparatus at spinal cord level.

If, however, we accept the present day views on the mechanism of action of the tetanus toxin, according to which this exerts its selective effect on motor neurons [8, 9, 12, 19], it is then difficult to explain the phenomenon, since motor neurons represent the final common path of reflex arcs. An increase of general reflex excitability, which might be associated with the generalized involvement of motor neurons, would obviously be of considerable importance in connection with the phenomenon described, since the latter develops only during the stage of geralized tetanus. However, this mechanism alone cannot be responsible for a selective facilitation of the development of convulsions caused by stimulating the tetanized limb.

Evidently the cause of the phenomenon must lie in the changed functional condition of those limbs of the reflex arc which receive and transform the afferent impulses entering the spinal cord via the dorsal roots and which transmit them to different levels of the central nervous system.



Electrical activity in muscles of the posterior group of the right thigh (upper trace in all oscillograms) and in the back muscles of the right side (lowermost curve in all oscillograms) in rats with spinal cord section; excitation was caused by pressure on the left foot (top two traces) and right foot (third trace). Time marker — 20 mseconds. Displacement of the time marker indicates period of applying pressure to the foot. (Second trace represents continuation of first). Experiment on 12/18 1957. Rat No. 2.

Descriptions have recently appeared [11, 13, 14, 16] which indicate the presence of special regulatory mechanisms determing the extent of the afferent stimulation to the cord; control over these mechanisms is exerted by inhibitory processes originating in the internuncial neurons of the posterior horns of the spinal cord. It is probable that in the development of tetanus such regulatory mechanisms are disturbed, and that as a result the whole of the afferent stimulation from the "tetanized" limb enters the spinal cord unchanged. At the same time, the possibility cannot be excluded that at an early stage in generalized tetanus there is also reciprocal inhibition of other forms of afferent stimulation (for instance, that from the opposite side), and that this accentuates the difference between the two sides. Subsequently, during the development of generalized tetanus and the destruction of all inhibitory processes, the asymmetry becomes somewhat reduced.

Also, the type of electrical activity developed, the long duration of the after-effect, the gradual decay of the excitation with separate repeated bursts, and the very high intensity of the reaction in response to comparatively weak stimulation, all suggest that an important part must be played by the large number of internuncial neurons of the cord which could bring about a prolonged increased stimulation of the motor neurons.

At present it is difficult to say which neurons play the part of "dispatching stations", and whether they lie in the substantia gelatinosa of the cord [20] or in the grey matter [10] where they would appear to act as collectors of different stimuli [15]. This problem will be the subject of further investigation.

From the neurological standpoint, it is clear that in the development of ascending generalized tetanus, a new form of central relationships are involved. The group of internuncial neurons on the "tetanized" side act as "dispatching stations" and form new functional relationships with the rest of the cord, being enabled, as it were, to send on stimulations unrestricted to all levels. The phenomenon described may have something in common with other forms of functional connections, for instance Ukhtomskii's dominance [7], Wedensky's hysteriosis* [1-4] and others); at the same time, however, the phenomenon differs from these others by special features which emphasize its distinct nature and allow it to be distinguished from many other neurological phenomena.

SUMMARY

Spread of the excitation along the spinal cord was studied in white rats in ascending general tetanus. The special feature of this phenomenon is that the excitation becomes generalized completely (in some cases only) on stimulating the limb into which the lethal dose of toxin had been injected. The clinical effect takes the form of a convulsive attack with characteristic features of spasticity. High division of the spinal cord had no appreciable effect on the phenomenon.

The same effect was observed on stimulating the central ends of the cut dorsal roots on the side of the toxin injection.

Synchronization of the bursts of excitation occurred in remote groups of motor neurons located in different segements of the cord.

The part played by the various types of internuncial and motor neurons of the spinal cord is discussed. It is suggested that neurons functioning as "dispatching stations" may play a special part.

LITERATURE CITED

[1] E. Sh. Airapet'yants, Transactions of the VMMA (Naval Medical Academy)*, volume 17, p. 54 (1949).

[2] O. A. Andriainen, Reports of the AN SSSR*, volume 70, No. 2, p. 331-334 (1950).

[3] N. E. Vvedenskii, Russkii vrach, No. 22, 1912 (Complete Collected Works*, volume IV, p. 325 (1953)).

[4] I. A. Vetyukov, Transactions of the VMMA (Naval Medical Academy)*, volume 17, p. 95 (1949).

[5] G. N. Kryzhanovskii, Byull, éksptl. biol. i med., No. 12, pp. 43-51 (1957).

[6] G. N. Kryzhanovskii, in book: Modern Problems of Nervism in Physiology and Pathology*. Moscow, 1958, p. 582.

[7] A. A. Ukhtomskii, The Nature of Dominance,** Complete Collected Works, volume 1, 1950.

[8] J. J. Abel, W. M. Firor and W. Chalian, Bull. Johns Hopkins Hosp., 1938, v. 63, p. 373.

*Name not verified.

**In Russian.

[9] V. B. Brooks, D. R. Curtis and G. C. Eccles, J. Physiol., 1957, v.135, p.655.

[10] S. Cajal-Ramon, Histologie du systeme nerveuse de l'homme et des vertebres, 1952, t. 1, p. 532.

[11] G. P. Couch and G. M. Austin, J. Neurophysiol., 1958, v.21, N. 3, p. 217.

[12] U. Friedemann, A. Hollander and G. M. Tarlov, J. Immunol., 1941, v. 40, N. 3, p. 325.

[13] K. E. Hagbarth and D. G. Kerr, B. J. Neurophysiol., 1954, v. 17, p. 295.

[14] F. Kleyntjens, K. Koizumi and C. Brooks, Mc., C. A. M. A. Arch. Neurology and Psychiatry, 1955, v. 73, N. 4, p. 425.

[15] C. M. Kolmodia, Acta physiol. scand., 1957, v. 40, Suppl. 139, Stokholm, 89, pp.

[16] U. T. Lindblom and G. O. Ottosson, Acta physiol. scand., 1956, v. 35, b. 3-4, p. 203.

[17] D. B. Lindsley, L. H. Schreiner and H. W. Magoun, J. Neurophysiol., 1949, v. 12, N. 3, p. 197.

[18] H. W. Magoun and R. Rhines, Spasticity; The Stretch Reflex and Extrapyramidal Systems (Springfield: C. Thomas, 1947).

[19] H. Meyer and F. Raasom, Arch. exper. Pathol and Pharmak., 1903, Bd. 49, H. 6, S. 369.

[20] A. A. Pearson, A. M. A. Arch. Neurol. and Psychiatry 1952, v. 68, N. 4, p. 515-529.

[21] L. H. Schreiner, D. B. Lidsley and H. W. Magoun, J. Neurophysiol., 1949, v. 12, N. 3, p. 207.

[22] A. A. Ward Jr. J. Neurophysiol., 1947, v. 10, N. 2, p. 89.

AN ELECTROPHYSIOLOGICAL STUDY OF DIFFERENT PARTS OF THE NERVOUS SYSTEM IN SHOCK

V. S. Sheveleva

From the I. P. Pavlov Institute of Physiology (Director – Academician K. M. Bykov, AN SSSR, Leningrad

(Received January 28, 1959. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

It has been shown both clinically and experimentally that there are many different causes of shock, and these may include operative measures, damage to the internal organs or limbs, injection of chemical substances, the action of ionizing radiation, or electrical nervous stimulation.

There are two phases – the excitatory and the depressive. They are shown by an initial increase in autonomic and somatic reactions which is followed by a period of decreased reactivity.

In the pathogenesis of shock, three factors of fundamental importance must be considered, and these are traumatic toxemia, loss of blood plasma, and pain.

Most Russian clinicians and physiologists [1-6, 8, 11-13, 15, 16] consider that shock is fundamentally a reflex mechanism resulting from a parabiotic condition of the central nervous system.

It is well known that extremely intense painful stimulation, such as occurs in damage to the limbs or internal organs, even when there is no great blood loss, may cause grave disorders in the central nervous system. Shock can also be caused by electrical stimulation of afferent nerves when there is no direct damage to the tissue and no production of toxic substances.

It was possible to follow the electrophysiological changes at different levels in the nervous system during the development of shock, and to form a concept of the primary cause of the resulting trophic disturbances.

METHOD

Shock was induced by painful stimulation applied to the intero- and exteroceptors, or by exposure to ionizing radiation.

The experiments were carried out on unanesthetized rabbits; unipolar and bipolar platinum electrodes were inserted into the cortex, hypothalamus, cerebellum, and preganglionic cervical sympathetic roots 1-2 weeks before inducing shock. In this way it was possible to make continuous observations before stimulation and to make continuous electrical recordings of the activity of these parts. In acute preparations, before applying the stimulation, electrodes were also placed in the superior cervical sympathetic ganglion and vagus.

Simultaneous recording of the potentials in the different parts of the nervous system was made using a six-channel oscillograph.

In one set of experiments, recordings of the electrical activity at the different levels in the nervous system were made during painful stimulation of the internal organs or limbs, done by pulling sharply on the mesentery of the small intestine, or by pressure applied to the limbs.

In another set of experiments, the changes were recorded during the exposure to ionizing radiation. Radiation was applied once to the surface of the belly (the head being screened), under the following conditions: voltage

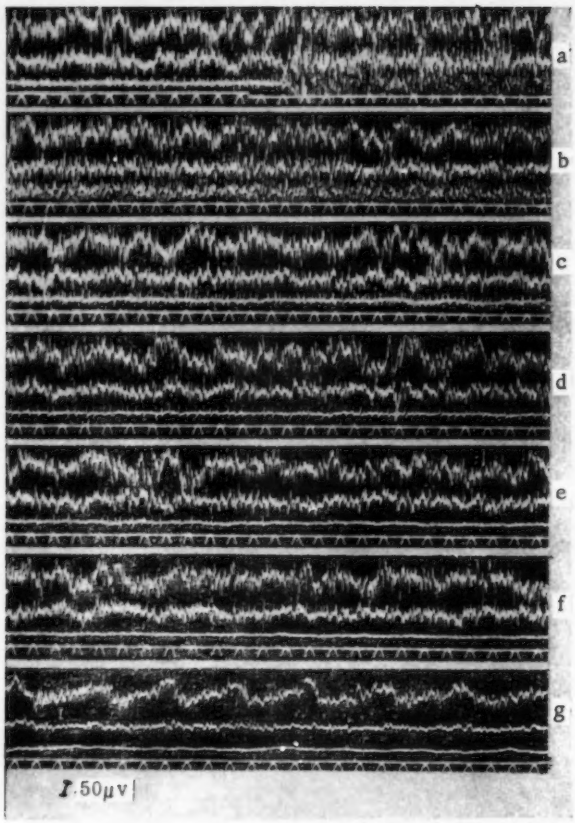


Fig. 1. Changes in electrical potentials recorded from different parts of the nervous system during continuous stimulation of the intestine. a) initial level of "spontaneous" activity, and beginning of stimulation; b, c, d, e, f, g, – ditto during stimulation. Curves, from above downwards: potentials from cortex, hypothalamus, superior cervical sympathetic ganglion; stimulus marker; time marker (0.1 second).

178 kv, current 10 ma, focusing distance 60 cm, filter 0.5 mm Cu and 1 mm Al, dose rate 12.2 r per minute, dose in air 9.5 r, and radiation dose 1200 r.

RESULTS

It has been shown experimentally that immediately after application of a painful stimulation, there is a burst of excitation which spreads reflexly to all parts of the nervous system (Fig. 1, a, b, c). However, owing to the different rates of propagation of the nervous impulses at the different levels and in the different parts of the nervous system, the period of abnormally high excitation is immediately followed by one of inhibition [17].

The inhibition develops first in the ganglia of the sympathetic system where the least labile synaptic connections in the descending reflex pathway are found. This effect is shown by a disturbance in the transmission of impulses through the superior cervical sympathetic ganglion occurring in the first 10-15 minutes (Fig. 1, d, e).

After the activity of the peripheral part of the sympathetic system has been reduced, as the painful stimulation continues there is a reduction also in the activity of the hypothalamic autonomic centers. This is shown

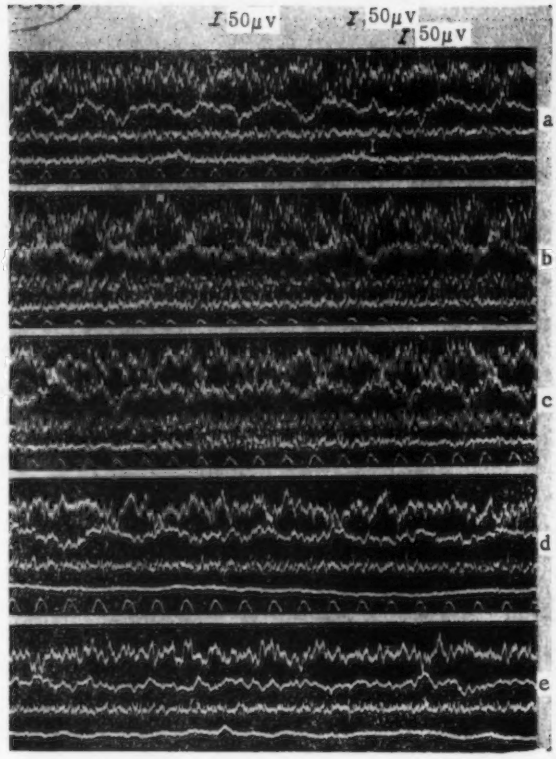


Fig. 2. Change in potentials recorded from different parts of the nervous system during exposure to ionizing radiation. a) background level; b, c) after exposure to dose of 400 r; d, e) after a dose of 1200 r. Curves, from above downwards: potentials from cortex, hypothalamus, cerebellum, superior cervical sympathetic ganglion; time marker — (0.1 second).

by a greater reduction in the electrical potentials of the hypothalamic area in comparsion with those of the cortex, where the potentials are still increased under the influence of impulses arriving from the periphery (Fig. 1, f). However, as the potentials in the hypothalamic area continue to fall, those in the cortex become reduced as well (Fig. 1, g). This last change is no doubt due to disturbance of the blood supply to the brain and changes in the oxidation-reduction processes associated with sympathetic failure. Simultaneous recording of the blood pressure in the common carotid artery and respiration showed that immediately after failure of synaptic transmission in the sympathetic ganglia, there is a fall in blood pressure and a sharp change in respiration rate.

The sequence of changes established for the different levels of the nervous system in traumatic shock also holds for shock caused by other kinds of stimuli, including large doses of ionizing radiation.

With large radiation doses, it was also found that when the animal was irradiated with a dose of 800-1200 r, immediately after the first increase in the potentials recorded (with dose of 100-200 r), marked changes in excitability occurred in the different parts of the nervous system. First there was an inhibition in the sympathetic ganglia, i.e., in the peripheral portion of the sympathetic nervous system (Fig. 2, c, d). Inhibition then occurred in the hypothalamus and cerebellum. During this period there was a gradual reduction in the potentials recorded from the cortex (Fig. 2, d, e).

Normally, central nervous trophic control is mediated via a complex sequence of nervous and endocrine mechanisms. The flow of impulses from the exteroceptors passes to the central nervous system and reaches the cells of the cortex where the impulses become added to those from the interoceptors. In response to stimulation of

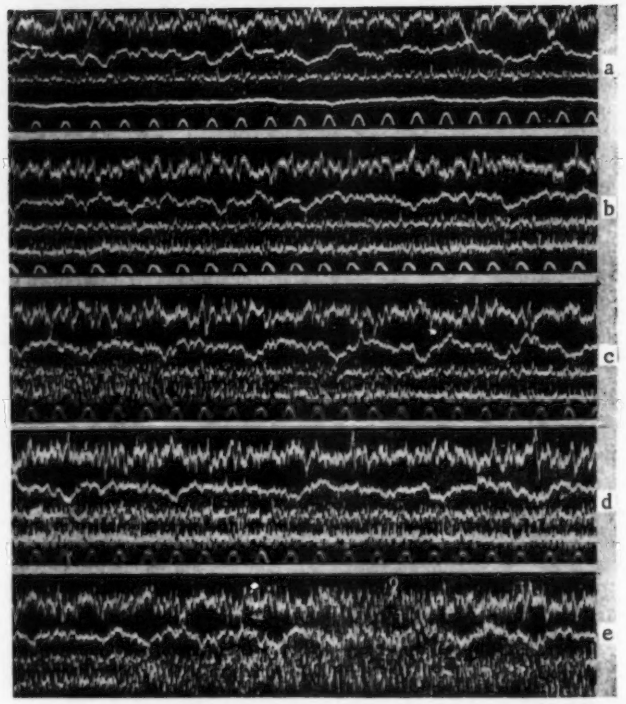


Fig. 3. Restoration of electrical activity in different parts of the nervous system by the mixture of drugs described in the text and ephedrine which remove the effect of the previously administered 1200 r dose of ionizing radiation. a) electrical activity before injecting the drugs, b) after injection; c, d, e) after an additional injection of ephedrine. Curves, from above downwards: potentials from cerebral cortex, hypothalamus, cerebellum, and superior cervical sympathetic ganglion; time marker (0.1 second).

cortical cells, impulses leave the cortex and pass peripherally to the effector organs. Nervous structures at lower levels now become reflexly excited. Impulses forming part of the reflex are transmitted along sympathetic neurones, and are concerned in the regulation of vital processes, including mobilization of liver glycogen, increasing the working capacity of muscles, redistribution of blood, maintenance of blood pressure, in thermoregulation, and in hormonal control [9, 10, 14]. When extremely strong stimuli are applied, the excessive excitation which is followed by parabiotic inhibition in the sympathetic nervous system stops the transmission of impulses from the center to the periphery.

The cessation of flow of impulses through the sympathetic system brings to an end the trophic control exerted by the central nervous system which normally brings about the mobilization of carbohydrate reserves and so maintains the energy balance, particularly when it is disturbed by extraneous stimuli. Failure of sympathetic transmission represents the onset of shock, which is followed by the characteristic autonomic and somatic reactions.

Further evidence that the shock is initiated by sympathetic disturbance is afforded by the fact that trophic changes due to shock, either in the excitatory or depressive phases, has been shown by many special investigations [3, 15] to be directly comparable with the changes which have frequently been encountered in physiological experiments on sympathectomy.

In shock, a kind of functional sympathectomy occurs through the complete cessation of impulses in the peripheral or ganglionic portion of the sympathetic system and a gradual reduction in the number of impulses in

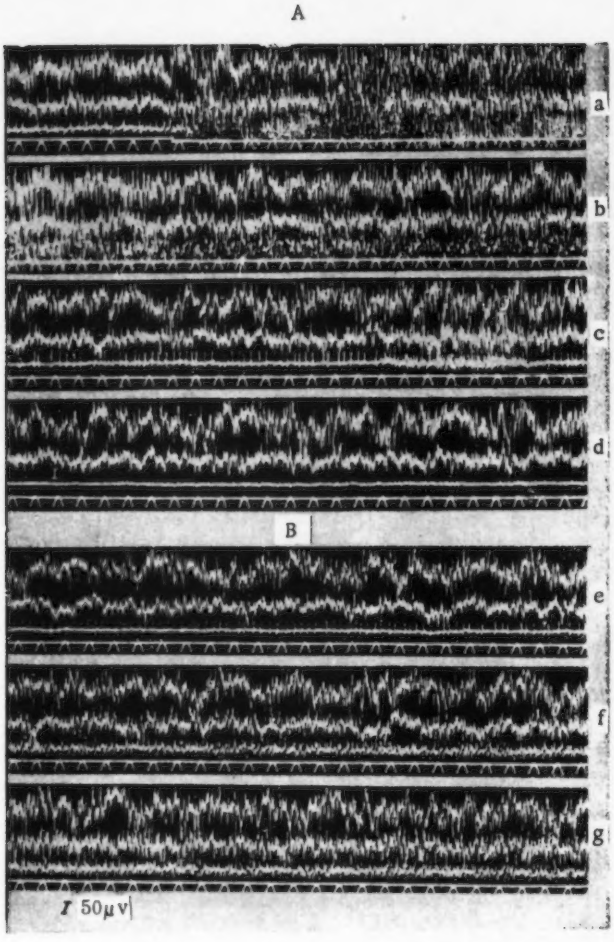


Fig. 4. Electrical activity of different parts of the nervous system during stimulation of intestinal receptors. A) before giving drugs: a) background level of electrical activity and beginning of stimulation (shown by drop in bottom line); b, c, d) continuous stimulation; B) after administering mixture of drugs (1 ml/kg); e, f, g) during continuous stimulation. Curves, from above downwards: potentials from cerebral cortex, hypothalamus, and superior cervical sympathetic ganglion. Time marker (0.1 second).

in the central parts of the system, i.e. , in the hypothalamus and cerebellum. Besides the metabolic disturbances, there is also an inhibition of the cells of the cerebral cortex which are under the direct influence of afferent impulses and which require energy to expend, which must be made good by the supply of appropriate material, in order to carry out reflex transmission to other parts of the nervous system. When the sympathetic system fails, this process is no longer possible and the excitatory condition of the cells of the central nervous system is replaced by one of inhibition.

Because the fundamental phenomenon underlying the condition of shock is the cessation of sympathetic synaptic transmission, we thought it advantageous to combat shock by using a complex preparation prepared by us for intravenous injection and consisting of a number of substances which stimulate the oxidation-reduction processes in nerve cells; these substances are thiamine, riboflavin, nicotinic acid, ascorbic acid, calcium chloride, alcohol, and glucose.

In this way we hoped to increase the lability of nerve cells in the lower levels of the nervous system and so to equalize the rate of transmission of impulses in the different parts of the nervous system. For this purpose, besides administering the mixture of drugs just described in order to stimulate the autonomic centers we also used ephedrine, given either intramuscularly or intravenously.

It was found that injection of 1-2 ml per kg weight of our preparation into rabbits in the inhibitory condition caused a restoration of the transmission of impulses. This took place independently of whether the parabiotic inhibition was induced by the action of extreme painful stimulation or by ionizing radiation. Figure 3 represents the direct continuation of Fig. 2, and it can be seen that after irradiation with 1200 r there was a complete blockage of impulses in the sympathetic ganglia and a marked reduction in hypothalamic and cerebellar potentials; intravenous injection of our preparation caused a rapid return of synaptic sympathetic transmission (Fig. 3, b). When an intramuscular injection of 0.5 ml of 5% ephedrine was also given, there was a further increase in the amplitude of the potentials recorded from the sympathetic ganglion, and from the hypothalamus and cerebellum (Fig. 3, c, d). At the same time there was a gradual increase in the amplitude and frequency of the impulses in the cerebral cortex (Fig. 3, d, e).

The stimulating effect of the mixture of drugs and of ephedrine on the electrical activity of the nervous system was observed during the application of painful stimuli to the intestine. This can be seen from Fig. 4, which shows simultaneous records from the cortex, hypothalamus, and a sympathetic ganglion. By injecting the mixture of drugs intravenously during the inhibition of the sympathetic system (Fig. 4, d), so increasing the lability of the cells of the sympathetic ganglia, it was possible to restore the transmission of excitation in the sympathetic system so that the parabiotic condition of the central nervous system failed to develop despite a continuation of the painful stimulation (Fig. 3, d). As the synaptic transmission in the sympathetic system returned, under the influence of the drugs, the blood pressure returned to its initial value, and the respiratory rhythm was re-established.

The increased sympathetic activity, by restoring the blood pressure, improved the circulation, and so made possible the mobilization of energy reserves. This in turn favored the return of synaptic transmission in all parts of the nervous system and increased the lability of all nervous cells, including those of the cerebral cortex, which in turn responded reflexly to impulses derived from external stimuli to send further trains of nervous impulses to cells of the autonomic centers, thus raising the level of their activity. This latter effect in turn resulted in the mobilization of sufficient energy and hormonal reserves to counteract the trophic disturbances induced by the excessive stimulation, so bringing about conditions which enabled the animal to emerge from the condition of shock, and the central nervous system once more to react to external stimuli.

This study of electrical activity in different parts of the nervous system during the action of extreme levels of stimulation has shown that the descending portion of the inhibitory reflex develops primarily in the peripheral sympathetic nervous system. By blocking the transmission of impulses in the sympathetic ganglia, central trophic control of tissue is eliminated, and conditions develop in which the shock syndrome appears.

SUMMARY

Electrical recordings were made of changes occurring at different levels of the nervous system during the development of shock.

In response to the action of the extremely strong stimuli, inhibition developed primarily in the peripheral portion of the sympathetic nervous system.

As a result of the blockage of impulses in the sympathetic ganglia, central nervous trophic control over the tissues was eliminated, and conditions created which led to the development of shock.

LITERATURE CITED

[1] E. A. Asratyan, Outlines of the Etiology, Pathology, and Therapy of Traumatic Shock*. Moscow, 1945.

[2] V. V. Babuk, Traumatic Shock and Gross Hemorrhage*, Minsk, 1953.

[3] S. I. Banaitis, Traumatic Shock in Laboratory Experiments, Clinical Practice, and Field Surgery*. Kaunas, 1948.

[4] A. A. Bogomolets, Transactions of the Conference on Problems of Shock*. Kiev, 1937, p. 5.

* In Russian.

[5] N. I. Burdenko, Klin. med., volume 16, issue 2, p. 135 (1936).

[6] L. L. Vasil'ev and D. A. Lapitskii, Research Reports of Leningrad State University*, Biological Series, Leningrad, 1944, issue 12, p. 114.

[7] N. E. Wedensky, Excitation, Inhibition and Anesthesia*. Leningrad, 1953.

[8] A. V. Visnevskii, Collected Works*, Moscow, 1950, volume 3.

[9] L. A. Orbeli, Izv. Nauchn., issled. in-ta im. Lesgafta, 1923, volume 6, p. 197.

[10] L. A. Orbeli, Lectures on the Physiology of the Nervous System*, Moscow–Leningrad, 1938.

[11] L. A. Orbeli, in book: Military Medicine, Collection*, Moscow–Leningrad, 1944, p. 5.

[12] L. A. Orbeli, in book: Military Medicine, Collection*, Moscow–Leningrad, 1945, issue 2, p. 3.

[13] L. A. Orbeli, in book: Military Medicine, Collection*, Moscow–Leningrad, 1946, issue 3, p. 3.

[14] L. A. Orbeli, Problems of Higher Nervous Activity*, Moscow–Leningrad, 1949.

[15] I. R. Petrov, Shock and Collapse*, Leningrad, 1947.

[16] I. R. Petrov, in book: Problems of the Physiology of the Central Nervous System*, Moscow–Leningrad, 1957, p. 447.

[17] V. S. Sheveleva, Fiziol. zhurn. SSSR, volume 44, No. 9, p. 882 (1958).

* In Russian.

RELATION OF SPLENIC ACTIVITY TO THAT OF OTHER ORGANS

COMMUNICATION III. EFFECT OF DIATHERMY APPLIED TO SPLEEN AND OF SPLEEN EXTRACTS ON THE LIVER AND HEMOPOIETIC ORGANS DURING PATHOLOGICAL CONDITIONS

L. I. Geller

From the Ufimskii Scientific Research Institute of Hygiene and Occupational Diseases

(Received November 22, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

The effect of experimentally induced splenic disorders on the condition of many other organs, particularly the liver, has previously been described [1, 2]. It seemed worth while to investigate the influence of the spleen on experimentally induced disorders of the liver. A humoral regulatory influence exerted by the spleen on metabolic processes in the liver has been reported [3].

METHOD

We applied diathermal treatment to the spleen in the same way as is used in the treatment of endocrine organs by stimulation [8]. At the same time we used splenic extracts. One such extract was obtained from intact rabbits and white rats. To obtain a second kind of extract, rabbits and rats were first joined in pairs, using the method of cutaneous-muscular parabiosis [9] in which, during the operation for joining the animals, the spleen is removed from one of them; 10 days later, the spleen is removed from the second partner, and an extract prepared from it. The spleen of the second parabiont is always larger than that of the first and has hypertrophied to fulfill many humoral functions, and so may serve as a model of the so-called hyperactive spleen; the activity is increased but does not become abnormal, as happens in splenic disorders [16].

The extracts were prepared by a method which has been described in detail by Ungar [17] and by Karjala and his coworkers [15]. For intramuscular injection, a solution of 1mg per ml of the extract obtained in this way was made in peach oil. Diathermy was applied to the spleen with an apparatus giving a wavelength of 200 m at strength of 0.15 amp using electrodes 3 × 3 cm for the rabbit and 1.5 × 1.5 cm for the rat; the treatment was continued for 15 minutes.

The particular liver disorder selected was toxic hepatitis induced by subcutaneous injection of carbon tetrachloride; in rabbits the dose was 0.5 ml per kg 4 times per day, while that for white rats was 0.3 ml per 100 g body weight, given 3 times daily.

Diathermy was started at the same time that the first injection of carbon tetrachloride was given, and was continued daily for 9 days in the case of the rabbits, and for 7 days for the white rats. Injection of 1 ml of an oily solution of splenic extract per kg body weight were given intramuscularly daily for 9 days, beginning at the time of the first carbon tetrachloride injection; the white rats received 0.2 ml per 100 g body weight daily for 7 days, beginning at the same time as the other injection.

The following measurements were made to determine the condition of the liver: changes in the blood sugar induced by adrenalin, total blood serum protein and protein fractions, liver glycogen and fat, uptake of sulfur-labeled methionine by liver protein, and the ability of liver sections to bind phenol and to oxidize tyrosine.

TABLE 1

Effect of Diathermy of the Spleen and Splenic Extracts on the Protein Content and Protein Fractions in the Blood Serum and Figures Showing the Effect of Adrenalin Injections Given to Rabbits Suffering from Toxic Hepatitis

No. of group of rabbits	Description of group	No. of animals	Protein (in g %)	Albumin (in %)	Globulin (in %)			A/G	Increase of blood sugar (mg %) during 4-hour test with adrenalin	
					α	β	γ		maximum	average
1	Intact	10	6.7±0.3	60.8±3.1	9.1±0.4	13.9±2 2	16.2±3.0	1.5±0.06	321±24.7	258±14.5
2	Toxic hepatitis	10	7.3±0.3	46.2±4.5	10.2±1.3	20.3±4,1	23.3±4.6	0.9±0.02	192±19.3	157±17.5
3	Toxic hepatitis+ diathermy to spleen	10	7.0±0.2	54.7±2.9	10.3±0.3	15.8±2.2	19.2±1.6	1.2±0.02	253±22.1	193±15.8
4	Toxic hepatitis+ extract of normal rabbit spleen	8	7.1±0.2	50.1±3.2	11.2±0.4	16.9±2.4	21.8±1.8	1.0±0.04	229±17.4	178±11.7
5	Toxic hepatitis + extract of hyperactive rabbit spleen	10	6.9±0.3	56.2±3.5	9.3±0.7	15.6±3.0	18.9±1.9	1.2±0.05	271±15.7	201±13.3
6	Toxic hepatitis + extract of hyperactive white rat spleen	10	7.0±0.4	55.9±3.0	8.8±0.5	16.0±2.8	19.3±2.1	1.2±0.04	262±18.2	196±18.9

In all the tables average values are given and root mean square deviations for each group.

TABLE 2

Effect of Splenic Diathermy and Splenic Extracts on the Uptake of Sulfur Labeled Methionine by Liver Protein and on the Ability of the Liver to Bind Phenols and to Oxidize Tyrosine in White Rats Suffering from Toxic Hepatitis

No. of group of animals	Description of group	Number of animals	Percentage uptake of methionine by liver protein	Relative activity	Binding of phenols (as % of amount injected)	Oxidation of tyrosine (in % of amount injected)
1	Intact	10	8.1 ± 0.4	0.61 ± 0.03	26.4 ± 1.7	42.7 ± 2.5
2	Toxic hepatitis	10	6.5 ± 0.4	0.39 ± 0.07	9.3 ± 1.1	20.2 ± 1.6
3	Toxic hepatitis + diathermy to spleen	10	7.6 ± 0.3	0.51 ± 0.05	15.7 ± 1.3	29.7 ± 2.2
4	Toxic hepatitis + extract of normal rat spleen	10	7.0 ± 0.2	0.45 ± 0.06	12.3 ± 1.0	24.4 ± 2.1
5	Toxic hepatitis + extract of hyperactive rat spleen	10	7.6 ± 0.3	0.54 ± 0.06	15.9 ± 1.8	30.1 ± 1.8
6	Toxic hepatitis + extract of hyperactive rabbit spleen	10	7.8 ± 0.3	0.52 ± 0.03	16.8 ± 1.5	27.1 ± 1.5

TABLE 3

Effect of Diathermy to Spleen and Splenic Extracts on Glycogen and Fat Content of Liver in Rabbits and White Rats Suffering from Toxic Hepatitis

No. of group of animals	Description of group	Rabbits			White rats		
		number of animals	glycogen (in g %)	fat (in g % of dry substance)	number of animals	glycogen (in g %)	fat (in g % of dry substance)
1	Intact	10	2.4 ± 0.5	8.7 ± 0.6	10	2.7 ± 0.3	11.8 ± 0.8
2	Toxic hepatitis	10	0.8 ± 0.2	18.9 ± 0.7	10	0.5 ± 0.1	27.6 ± 1.2
3	Toxic hepatitis + diathermy to spleen	8	1.3 ± 0.3	13.1 ± 0.8	10	1.2 ± 0.2	20.9 ± 0.8
4	Toxic hepatitis + extract of normal rabbit spleen	8	0.9 ± 0.4	15.9 ± 0.5	10	0.7 ± 0.1	23.3 ± 1.6
5	Toxic hepatitis + extract of normal rat spleen	8	1.0 ± 0.2	15.4 ± 0.6	10	0.9 ± 0.1	23.8 ± 1.9
6	Toxic hepatitis + extract of hyperactive rabbit spleen	10	1.4 ± 0.3	12.2 ± 0.7	10	1.1 ± 0.1	18.7 ± 1.2
7	Toxic hepatitis + extract of hyperactive rat spleen	10	1.3 ± 0.6	12.7 ± 0.5	10	1.4 ± 0.91	19.5 ± 1.4

TABLE 4

Effect of Diathermy to the Spleen and Splenic Extracts on Blood Indices in Benzene Poisoning in Rabbits

Blood index	Description of group			
	benzene without therapy (12 rabbits)	benzene + diathermy to spleen (12 rabbits)	benzene + extract of normal spleen (10 rabbits)	benzene + extract no. 1 of hyperactive spleen (12 rabbits)
Leukocytes (in thousands) per mm^3; initial count	9.3 ± 0.4	9.0 ± 0.7	9.6 ± 0.4	9.2 ± 0.2
9-10th day after first benzene injection	2.7 ± 0.4	4.5 ± 0.7	4.2 ± 0.3	4.3 ± 0.6
26-27th day after first benzene injection	5.4 ± 0.8	8.6 ± 0.5	5.9 ± 0.4	8.9 ± 0.6
Granulocytes (in thousands) per mm^3; initial count	3.2 ± 0.3	3.4 ± 0.1	3.5 ± 0.6	3.4 ± 0.5
9-10th day after first benzene injection	1.4 ± 0.1	1.8 ± 0.2	1.3 ± 0.2	2.0 ± 0.4
26-27th day after first benzene injection	2.1 ± 0.2	2.9 ± 0.3	2.2 ± 0.3	3.0 ± 0.3

Adrenalin was given to rabbits as a subcutaneous injection of 0.3 ml per kg body weight of a 1 : 1000 solution. Total protein was determined using a refractometer, and the protein fractions estimated by paper electrophoresis. Glycogen was determined by Cori's method as modified by Brand, and the total liver lipids by extracting the dried liver tissue with dichlorethane in a Soxhlet apparatus [10]. Sulfur labeled methionine was injected intraperitoneally 24 hours before killing, to give 5000-6000 impulses per minute per g body weight. To eliminate the effect of possible changes in the permeability of liver tissue to the isotope, a determination was made of the activity of the protein relative to that of the protein in 1 g of tissue and to the activity of 1 g of whole tissue. The binding of phenol and the oxidation of tyrosine by the liver sections was measured by the usual methods [6, 7].

RESULTS

The results are shown in tables 1, 2, and 3.

The results shown in the tables indicate that in toxic hepatitis, diathermy applied to the spleen and hyperactive spleen extract exert a definitely favorable influence on the functional condition of the liver in all cases: there is an increased glycemic reaction to adrenalin, liver glycogen is raised, and the fat content reduced; there is a reduction in blood albumen and an increase in the globulin fraction, the antitoxic action of the liver is raised, as is also the ability of the liver to synthesize protein. The effect on these indices of extracts of normal spleen from intact animals is very much smaller.

In addition to the experiments whose results are given in the tables, we also carried out others in which we investigated the effect of splenic diathermy and the action of various splenic extracts on liver function (using the indices of the tests) in intact animals (without hepatitis); it was not possible to determine any consistent changes in these experiments, and the results have therefore not been included in the tables.

It seemed most interesting and important to us that the effect of the hyperactive spleen extract did not depend on whether it was prepared from an animals of the same species as that in which the hepatitis was induced or from the spleen of a different species. The results obtained confirm that the liver and spleen are functionally related, and give certain indications of how to make use of these correlations clinically.

Splenic diathermy and injection of splenic extracts were also used by us during a study of the myelo-lienal correlation under pathological conditions. The related functions of the bone marrow and spleen have frequently been studied by Soviet and foreign workers [5, 11, 12, 13].

TABLE 5

Effect on Rabbits of Diathermy to Spleen and of Splenic Extracts on Blood Indices after General X-ray Irradiation

Blood Index	Description of group				
	irradiation without therapy (14 rabbits)	irradiation + + diathermy to spleen (10 rabbits)	irradiation + + extract of normal spleen (10 rabbits)	irradiation Extract No. 1 of hyperactive spleen (15 rabbits)	irradiation + + Extract No. 1 of hyperactive spleen (15 rabbits)
Leukocytes (in thousands) per mm^3;					
initial count	9.1±0.6	9.4±0.5	9.3±0.8	8.9±0.8	9.3±0.4
10 days after irradiation	1.7±0.4	2.2±0.5	2.3±0.6	2.9±0.3	3.4±0.2
30 days after irradiation	6.2±0.8	8.8±0.4	8.0±0.4	8.9±0.5	9.1±0.6
Thrombocytes (in multiples of one hundred thousand) per mm^3; initial count	2.4±0.3	2.7±0.4	2.1±0.2	2.6±0.3	2.4±0.3
10 days after irradiation	0.6±0.8	1.0±0.1	0.8±0.08	1.0±0.1	1.2±0.1
30 days after irradiation	1.4±0.3	2.3±0.2	1.8±0.1	2.1±0.4	2.5±0.2
Young erythroblasts of bone marrow as % of total cells:					
10 days after irradiation	2.3±0.4	5.0±0.6	4.5±0.3	5.2±0.3	6.7±0.6
20 days after irradiation	7.1±0.5	13.2±1.5	10.9±0.8	11.3±1.6	14.8±1.5
Young granulocytes of bone marrow as % of total cells:					
10 days after irradiation	1.3±0.3	4.7±0.6	3.2±0.9	4.5±0.8	5.2±0.5
20 days after irradiation	3.0±0.6	10.8±1.2	6.9±1.1	10.1±0.6	12.1±1.6
Uptake of Fe^{59} per 1 ml erythrocytes (in thousands of impulses per minute):					
10 days after irradiation	0.6±0.05	2.9±0.2	1.3±0.1	2.7±0.2	2.5±0.3
20 days after irradiation	2.7±0.2	6.4±0.2	3.2±0.3	6.1±0.2	7.9±0.4

Our experiments were made on animals with benzene poisoning or suffering from the effects of ionizing radiation.

In planning the work, we had in mind both the theoretical importance of this correlation and its effects in pathological conditions, as well as the undoubted practical importance from the clinical standpoint. The important part played by the spleen in the pathogenesis of radiation sickness has been demonstrated by the investigations made in recent years [4, 11].

The experiments were carried out on rabbits weighing 1700-2400 g. Total x-ray irradiation was applied as follows: voltage 17 kv, current 20 mamp, focusing distance 90 cm, filters 0.5 mm Cu and 1 mm Al, power of dose 13.7-14.2 r/minute. The rabbits received a dose of 600 r. In the experiments with benzene poisoning 1 ml of this substance per kg weight was injected subcutaneously daily for 5 days. The composition of the blood and bone marrow was measured, the latter being aspirated from a hole trepanned in the femoral bone. In experiments with irradiation, we also determined the uptake of radioactive iron Fe^{59} in the erythrocytes, which gives an indication of the erythropoietic activity of the bone marrow (the isotope was injected intravenously in a dose of 5 microcuries per kg body weight).

Diathermy to the spleen was applied daily, by the method described above, for 2 weeks after irradiating or after the first benzene injection. In the radiation experiments, besides the extracts of hyperactive spleen used previously, which we will refer to in future as "No. 1", we also used another heterogenous splenic extract prepared as follows: the rabbits were joined as parabionts, after which one was irradiated with 800 r of x-rays, while the other was protected by a lead shield; one week later, the spleen was removed from the second (unirradiated) parabiont, and an extract of it made. Usually, the spleen of the unirradiated member of the pair has a far greater weight not only than that of its irradiated partner, but is also heavier than that of a normal rabbit. Our provisional hypothesis was that the spleen of the unirradiated parabiont, as in the case of parabiosis with a splenectomized partner, increases its activity also by way of compensation when joined to an irradiated partner. The extract obtained in this way is referred to as "Extract No. 2". The extracts were injected intramuscularly as 1 ml of an oily solution per kg weight; they were given daily for 2 weeks, starting on the day of the irradiation or of the first benzene injection.

The results of measurements on the changes in the blood indices as affected by benzene and x-ray irradiation are shown in Tables 4 and 5.

From the results shown in the tables it can be seen that in benzene poisoning and in radiation sickness diathermy applied to the spleen, and extracts of hyperactive spleen have a very definitely favorable influence on the blood indices; thus, in the rabbits treated by these methods there was a smaller reduction in the number of formed elements in the peripheral blood, and a smaller reduction in the formation of new granulocytes and erythrocytes in the bone marrow than in the untreated animals, the difference being statistically significant.

The improvement in hemopoiesis effected by splenic diathermy and the injection of hyperactive splenic extract was particularly well shown in the period of early repair of the bone marrow. We were able to observe this phenomenon in both radiation sickness and benzene poisoning.

The results obtained and here presented extend our knowledge of the correlation between bone marrow and spleen and between liver and spleen under pathological conditions, and give real hope of the possibility of using these correlations clinically.

SUMMARY

Studies were made of the glycemic reaction to adrenalin, the protein content and protein fractions in the blood serum, the glycogen and fat contents of the liver, and the ability of the liver to take up sulfur-labeled methionine, to bind phenols, and to oxidize tyrosine. It was shown that diathermy applied to the spleen improves the condition of the liver in rabbits and white rats with hepatitis induced by carbon tetrachloride.

An analogous effect was achieved by an extract from an hyperactive spleen obtained by cutaneous-muscular union of pairs of animals as parabionts.

In a further set of experiments it was shown that splenic diathermy and extracts from a hyperactive spleen cause a marked improvement in peripheral blood and bone marrow indices in rabbits suffering from benzene poisoning or irradiation sickness.

LITERATURE CITED

[1] L. I. Geller, Byull. éksptl. biol. i med., volume 46, No. 11, p. 17 (1958).*

[2] L. I. Geller, Byull. éksptl. biol. i med., volume 47, No. 1, p. 35 (1959). *

[3] L. M. Gol'ber, Byull. éksptl. biol. i med., volume 25, issue 5, p. 396 (1948).

[4] Yu. M. Zaretskaya, Med. radiol., volume 3, No. 2, p. 81 (1958).

[5] S. I. Kaplanskaya, Byull. éksptl. biol. i med., volume 24, issue 3, p. 215 (1947).

[6] S. Kaplanskii and S. Kaplanskaya, Biokhimiya, volume 14, No. 2, p. 130 (1949).

[7] I. A. Kassirskii and G. A. Alekseev, Clinical Hematology**, Moscow, 1955.

[8] N. B. Medvedeva, Experimental Endocrinology**, Kiev, 1946, p. 54.

[9] L. R. Perel'man, Transactions of the Leningrad Medical Institute**, issue 6, p. 3 (1935).

* Original Russian pagination. See C. B. translation.

** In Russian.

[10] L. R. Perel'man, Regulation of Fat and Carbohydrate Metabolism* , Khar'kov, 1940.

[11] L. L. Faerman, Diseases of the Spleen* , Moscow-Leningrad, 1928.

[12] V. N. Chernigovskii and A. Ya. Yaroshevskii, Problems of the Nervous Regulation of the Blood System* , Moscow, 1953.

[13] L. Heilmeyer, Bibliotheca Haematologica, 1955, N. 3, S. 21.

[14] L. O. Jacobson et al., Proc. Soc. Exper. Biol. Med., 1949, v. 70, p. 740.

[15] S. A. Karjala and others. In the book: Splenin A. in Rheumatic Fever, Springfield, 1955, p. 75.

[16] E. Landa, Bibliotheca Haematologica, 1955, N. 3, S. 3.

[17] G. Ungar, Am. J. Physiol., 1951, v. 166, p. 340.

* In Russian.

THE ACTION OF LOW TEMPERATURE AND IONIZING RADIATION ON THE SERUM PROTEINS AND PROTEIN FRACTIONS

S. A. Brailovskii and Yu. K. Ledentsov

From the Department of Biochemistry (Head — Prof. S. A. Brailovskii) of the Sverdlovsk Medical Institute (Director — Prof. A. F. Zverev)

(Received July 14, 1959. Presented by Active Member AMN SSSR V. N. Chernigovskii)

Research by many authors has shown that irradiation of animals leads to changes in the normal proportions of the protein fractions of the blood serum [1, 2, 4]. As a result of irradiation of animals by ionizing radiation from different sources, a fall in the albumin and a considerable increase in the globulin content take place, as a rule. Changes in the serum proteins of an animal subjected to irradiation during the local action of a low temperature, however, have received little attention in the literature. There are merely indications that the application of general cooling stimulates the protective properties of the animal against ionizing radiation [6, 7]. So far as the clinical features and the biochemical changes which accompany the combined action of low temperature and ionizing radiation are concerned, apart from the work of O. V. Rudenko [3] we have been unable to find any relevant data in the accessible literature.

The aim of the present research was to study, in experiments on rats, the changes in the total protein and the protein fractions of the blood serum in response to the action of local cooling against a background of marked radiation sickness.

EXPERIMENTAL METHOD

Experiments were conducted on male white rats weighing 120-160 g. The total number of animals was divided into four groups of 10 rats each: a control group, a group of animals exposed to the local action of low temperature only, a group of irradiated animals and a group exposed to the combined action of low temperature and irradiation.

The local action of low temperature was effected by placing the animals in an open glass chamber, the bottom of which was immersed in a cooling mixture (ice, salt, carbon dioxide snow) at a temperature of -30° for 4 hours. In view of the fact that the cooling chamber was open and the temperature of the air within was kept at +14°, a purely local effect of cold on the limbs supporting the animals on the floor of the chamber was observed. The body temperature of the rats was measured by means of a thermocouple and did not depart from the range 34-36°, while the temperature of the muscles of the cooled extremities fell to +15° and +20°. After 24 hours the action of the low temperature was shown by the appearance of 2nd-3rd degree frostbite of the hindlimbs and tail. Hyperemia of the skin appeared, and this soon acquired a cyanotic hue. Marked edema of the skin and subcutaneous cellular tissue, cyanosis, and small areas of darkening were later observed.

The animals were irradiated with an RUM-3 apparatus, with a filter of 0.5 mm Cu and 1 mm Al, a current strength of 10 ma, and voltage of 190 kv. The total dose of irradiation was 700 r.

The total serum protein was estimated refractometrically. The protein fractions of the blood were estimated by the method of electrophoresis on filter paper. Electrophoresis was carried out in a veronal-medinal buffer (pH = 8.6) for 20 hours. The electrophoregrams were stained with bromphenol blue, cut into strips 5 mm wide, and after extraction of the dye the protein fractions were estimated on the electrophotocolorimeter.

Changes in the Total Protein Content and the Protein Fractions of the Serum of Rats (in %) after the Action of a Low Temperature and Irradiation

Protein fraction	Control animals	Freezing	Irradiation	Irradiation + freezing
Total protein	7.4	7.3	7.0	5.8
Albumins	51.8	42.5	42.5	30.5
Globulins	48.2	57.5	57.5	69.6
α_1-globulins	6.0	8.0	6.3	13.9
α_2-globulins	7.5	12.7	12.0	13.5
β-globulins	20.7	27.7	21.8	26.8
γ-globulins	13.5	9.0	17.1	13.8
A/G ratio	1.07	0.7	0.7	0.4

The rats exposed to the local action of cold were sacrificed by decapitation 24 hours after cooling, and the total protein content of the serum and the proportions of the protein fractions were estimated.

EXPERIMENTAL RESULTS

From the figures in the table it can be seen that, in the group of animals exposed to the local action of cold, obvious changes took place in the proportions of the serum protein fractions, namely a decrease in the concentration of albumins and an increase in the globulins, mainly affecting the β- and α_2-globulin fractions. These changes in the serum protein fractions led to a fall in the A/G ratio to 0.7.

In animals exposed to a single dose of total irradiation with x-rays of absolutely lethal magnitude, the investigation was carried out on the 7th day after irradiation when radiation sickness was well developed. At this time the rats showed considerable leucopenia (1200-1500 leucocytes/mm^3), severe adynamia, refusal to take food, liquid stools, hemorrhages from the ears and lips, and loss of weight. These signs were accompanied by a fall in the total serum protein level and also by characteristic changes in the proportions of the individual protein fractions, namely a fall in the albumin and an increase in the α- and β-globulin fractions.

In the next series of experiments, animals previously irradiated were exposed to the action of a low temperature (on the 7th day after irradiation). The course of the frostbite in these rats showed certain distinctive features: the hyperemia of the skin at the points of application of the cold was less intense, the frostbitten limbs were less cyanotic, and formation of blisters took place over a much larger area and their contents were often blood-stained. The tendency towards necrosis was more marked and healing took place more slowly. In this group of animals the most significant changes were observed in the total serum protein concentration and in the proportions of the protein fractions. The total proteins fell to 5.8% as against 7.4% in the control group. The very considerable fall in the albumins (control – 51.8%, experiment – 30.5%) and the increase in the α_1-, α_2- and β-globulin fractions were noteworthy. The A/G ratio fell from 1.07 to 0.4.

The literature of recent years has contained a number of papers devoted to the investigation of the blood proteins in radiation sickness [1, 2, 5]. The authors have concluded that the fall in the albumin fraction of the serum proteins is connected with disturbances of synthesis of this fraction. V. M. Rodionov and his co-workers [2], for instance, consider that in radiation sickness the new formation of albumins is depressed or even completely arrested. There is still a possibility that albumins may leave the blood stream and enter the tissues as a result of the increased permeability of the vessel walls, which is especially likely when it is remembered that considerable morphological changes occur in the vessel walls in radiation sickness. The combination of the action of low temperature with that of radiation sickness led to a severer course of the disease and to correspondingly more pronounced disturbances of protein metabolism.

The mechanisms underlying the changes of a fall in the total protein concent and a sharp fall in the albumins with an increase in the α_1-, α_2- and β-globulins in response to the combined action of irradiation and local cooling have not yet been explained and further research in this direction is required.

SUMMARY

Experiments were performed on rats. The authors determined the changes in the total protein content of the blood serum and the correlation of the protein fractions in response to the local action of low temperature and irradiation of animals with a lethal dose of X-rays. The combined effect of these factors brings about hypoproteinemia and a change in the correlation of the protein fractions towards a reduction of albumin fractions and an increase of the α_1-, α_2- and β-globulins.

LITERATURE CITED

[1] I. I. Ivanov, V. S. Balabukha, E. F. Romantsev and T. A. Fedorova, Metabolism in Radiation Sickness, p. 97. Medgiz, 1956 [In Russian].

[2] V. M. Rodionov, V. D. Uspenskaya, O. G. Zamyatina, T. A. Grunt and V. Polyakova, Voprosy Med. Khim, No. 5, 327 (1958).

[3] O. V. Rudenko, Med. Radiol., No. 3, 34 (1959).

[4] S. G. Shukurin, Problems of Roentgenology and Oncology, Vol. 2, p. 235, Erevan, 1957 [In Russian].

[5] A. Chanutin and E. Gjessing, J. biol. chem., 1946, v. 165, No. 2, p. 421.

[6] J. B. Storer and L. H. Hempelmann, Am. J. Physiol., 1952, v. 171, No. 2, p. 341.

[7] F. Smith and M. Grenan, Science, 1951, 113, 2946, p. 688.

THE CORRELATION OF THE CONTENT OF SULFHYDRYL GROUPS WITH THE CEREBROSIDE METABOLISM OF THE BRAIN IN THE RABBIT

M. Sh. Promyslov and M. S. Morozova

From the Biochemistry Laboratory (Head – Prof. V. M. Rubel') of the Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. N. Chernigovskii) of the AMN SSSR, Moscow

(Received March 3, 1959. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In 1936 Thannhauser and Reichel [5] reported the discovery of the enzyme cerebrosidase in the brain, capable of splitting a molecule of galactose from the cerebrosides. According to these workers the enzyme is active only when high concentrations of compounds containing SH-groups are added to the incubation medium.

After analyzing these findings in connection with our previous discovery of a fall in the concentration of cerebrosides in the brain tissue of rabbits in general tetanus and strychnine poisoning [1], we suggested that this

Content of SH-Groups in the Brain of Rabbits (γ/g of Fresh Tissue)

Normal			Tetanus			Strychnine		
total	low molecular	protein	total	low molecular	protein	total	low molecular	protein
360	45	315	448	20	428	452	55	397
390	45	345	388	55	333	484	38	446
400	45	355	340	35	305	464	45	419
372	53	319	400	35	365	432	45	387
388	40	348	440	48	392	512	38	475
460	45	415	420	45	375	440	63	377
432	45	387	500	60	440	476	65	411
470	45	425	416	55	361	540	55	495
450	63	387	460	70	390	—	—	—
412	60	352	428	57	371	—	—	—
Mean values								
413,4	48 6	364,8	424,0	48,0	376,0	475,0	50,5	425,0

fall in the cerebroside content was associated with an increase in the activity of the cerebrosidase of the brain, and this, in turn, may have been due to an increase in the content of sulfhydryl groups.

In order to discover a possible correlation between the content of SH-groups and the concentration of cerebrosides in brain tissue, we determined the content of SH-groups in the brain tissue of normal rabbits and of animals suffering from general tetanus or receiving half a lethal dose of strychnine.

EXPERIMENTAL METHOD

Tetanus toxemia was induced by injection of a lethal dose of tetanus toxin into a muscle of the left hind-limb. The animals were sacrificed in a state of general tetanus on the 7th-8th day after the toxin was injected. Strychnine nitrate was injected subcutaneously into the rabbits, in a dose of 1 mg dissolved in 0.5 ml of distilled water. After injection of this dose, convulsions developed in 10-15 minutes and were repeated periodically for 50-60 minutes. At the end of this period the animals were decapitated and the brain was extracted and frozen in a mixture of dry ice and ether. The total content of SH-groups in the brain was estimated by the method of A. S. Tsiperovich and A. L. Loseva [3], and the content of low molecular SH-groups by the method of Grunert and Phillips [4]. The difference between the total SH-groups and the low molecular sulfhydryl groups gave the content of free SH-groups of the protein.

EXPERIMENTAL RESULTS

During the investigation of the brain tissue of the normal rabbits, considerable individual variations were found in the content of protein SH-groups (see table). Similar individual variations in the content of sulfhydryl groups of the brain were noted in the work of other authors [2, 5, 6]. As will be seen from the results in the table, the total, low molecular and protein SH-group contents in the brain of the rabbit in a condition of general tetanus were practically indistinguishable from the normal values.

After strychnine convulsions in a rabbit, the brain showed a small (16.4%) but statistically significant increase in the content of protein SH-groups. The content of low molecular sulfhydryl groups was the same as in normal animals.

Since a fall in the content of cerebrosides in the brain of rabbits was observed in both general tetanus and strychnine poisoning, but an increase in the content of sulfhydryl groups was found only in the latter case, it may be assumed that the fall in the cerebroside content in our experiments was not connected with the change in the content of SH-groups in the brain tissue. If the converse were true it would have been necessary to postulate that the mechanisms of the decrease in the content of cerebrosides in the brain of the animals in the two forms of poisoning described above, each accompanied by severe convulsions, were different, which seems unlikely.

SUMMARY

The author studied the content of SH-groups in the brain tissue of healthy rabbits and in animals in a condition of general tetanus or after the administration of 50% of the lethal dose of strychnine. The data obtained led to the conclusion that there is no direct connection between the activity of the cerebrosidase and the content of SH-groups in the brain.

LITERATURE CITED

[1] M. Sh. Promyslov, Biochemistry of the Nervous System, p. 323. Kiev, 1957 [In Russian].

[2] K. V. Savich and V. A. Yakovlev, Voprosy Med. Khim., 3, No. 2, 121 (1957).

[3] A. S. Tsiperovich and A. L. Loseva, Ukrain. Biokhim. Zhur., 20 No. 1, 106 (1948).

[4] R. R. Grunert and P. H. Phillips, Arch., Biochem., 1951, v. 30, p. 217.

[5] S. J. Thannhauser and M. J. Reichel, J. Biol. Chem., 1936, v. 113, p. 311.

[6] G. Ungar and D. V. Romano, Proc. Soc. Exper. Biol. and Med., 1958, v. 97, p. 324.

THE ROLE OF THE COLLOID IN THE HORMONE-FORMING FUNCTION OF THE THYROID GLAND

M. F. Merkulov

From the Department of Pharmacology (Head – Active Member AMN SSSR the late V. I. Skvortsov) of the N. I. Pirogov Second Moscow Medical Institute

(Received February 18, 1959. Presented by Active Member AMN SSSR V. I. Skvortsov)

The role of the individual structural formations of the thyroid gland in the synthesis of its hormones is one of the more important problems in the histophysiology of this organ. After it had been proved that the colloid of the thyroid gland consists mainly of organic compounds of iodine, the function of a peculiar hormonal depot was ascribed to it. As a result of this, the cells of the follicular epithelium were endowed with the ability to secrete products both into the blood stream and into the cavity of the follicle. When there was an increased demand of hormone by the body, the cells of the follicular epithelium were called upon to mobilize reserves of hormone from the colloid.

This view of the role of the colloid was shaken by the first autoradiographic investigations of the hormone-forming function of the thyroid gland, using radioactive iodine [10, 13, 15, 17]. It became clear from these investigations that it is not only the surplus but practically the whole of the iodine entering the body that passes through the colloid. These findings made it necessary to study the role of the colloid in hormone formation in greater detail.

Bearing in mind that the bulk of the colloid consist of iodothyroglobulin, and using the method of microautoradiography, we attempted to trace the fate of its various component parts in the structures of the thyroid gland. As an indicator of the path of the protein part of the iodothyroglobulin we used S^{35}-methionine. Methionine, together with cystine, accounts for about 7% of the total amino acid content of iodithyroglobulin, and roughly the same or slightly less of the total is accounted for by tyrosine, diiodotyrosine and thyroxin taken together [1, 4, 9]. As a metabolite directly concerned in the formation of the hormonal products of the thyroid gland, we used radioactive iodine.

In this research we tried to show which structures of the thyroid gland first fix these compounds in the protein-bound form, for this would give some idea of the place of synthesis of thyroglobulin and of the site of its iodization.

EXPERIMENTAL METHOD

Experiments were conducted on male white rats weighing from 150 to 200 g. The radioactive substances were injected intravenously in the following doses: NaI^{131} without carrier – from 10 to 600 μC/kg, S^{35}-methionine – from 300 to 5000 μC/kg. The animals were sacrificed by decapitation after various intervals of time: 10 seconds, 5 and 20 minutes and, finally, 1, 4, 24 and 72 hours after injection of the labeled preparations.

The thyroid gland of the animal was extracted en bloc with the trachea as quickly as possible after death and placed in absolute ethanol, cooled to 0°. After fixation, the tissue was passed through a series of xylols and embedded in paraffin wax. Serial sections of the organ, 4-5 μ in thickness, were placed on glass slides,

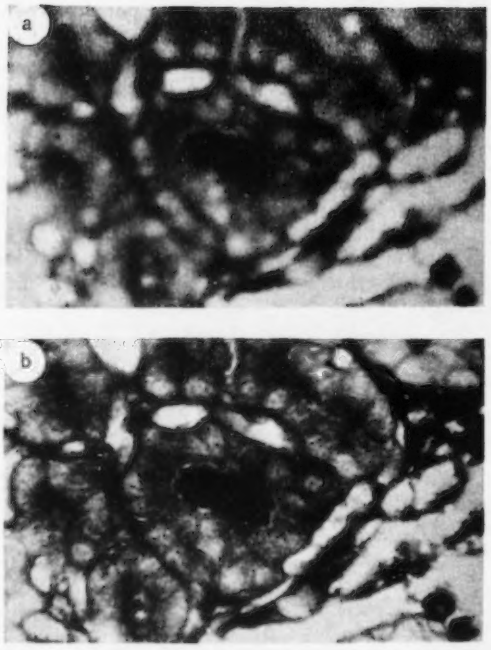


Fig. 1. Micro-autoradiograph of a follicle of the thyroid gland of a rat (the animal was sacrificed 10 seconds after the intravenous injection of 600 $\mu C/kg$ NaI^{131}). a) focused on the photographic emulsion. Concentration of the silver grains is observed over the colloid only; b) focused on the tissue ; "Azane" stained; magnification 40 x 10.

and after removal of the paraffin wax, were stained with hematoxylin-eosin or by the "Azane" method. Micro-autoradiographs were obtained by the method which we have described previously [3].

By this method of histological treatment of the material all the radioactive iodine compounds not combined with protein were completely washed from the tissues. We satisfied ourselves on this point by preliminary experiments in which we injected animals with mercazole, which blocks the iodization of thyroglobulin but does not affect the power of the thyroid gland to concentrate iodides from the plasma. Such treatment also washes from the tissues practically all the radioactive conversion products of S^{35}-methionine or the free labeled amino acid [6, 16]. In this way we recorded on micro-autoradiographs the distribution in the tissues of the protein-bound compounds of I^{131} and S^{35} alone.

EXPERIMENTAL RESULTS

Only 10 seconds after the intravenous injection of radioactive iodine, the thyroid gland contained well-defined amounts of protein-bound radioactive iodine compounds (Fig. 1), and the iodine was shown to be concentrated entirely in the cavity of the follicles — in the colloid. In sections of the follicles which were large in size, the radioactivity was sometimes distributed in the form of a closed circle along the border of the apical parts of the cells and the colloid. In sections in which, in the course of preparation, the colloid had become separated from the follicular epithelium, it was seen particularly clearly that the radioactivity was wholly bound up with the colloid. Sometimes the accumulation of protein-bound compounds of radioactive iodine in the colloid had the appearance in the sections of an open circle or semicircle. It is interesting to observe that at such an early stage as 10 seconds after the injection of radioactive iodine, it was not present in all the follicles in protein-bound form. The number of follicles containing radioactive thyroglobulin was 5-10 % of the remainder.

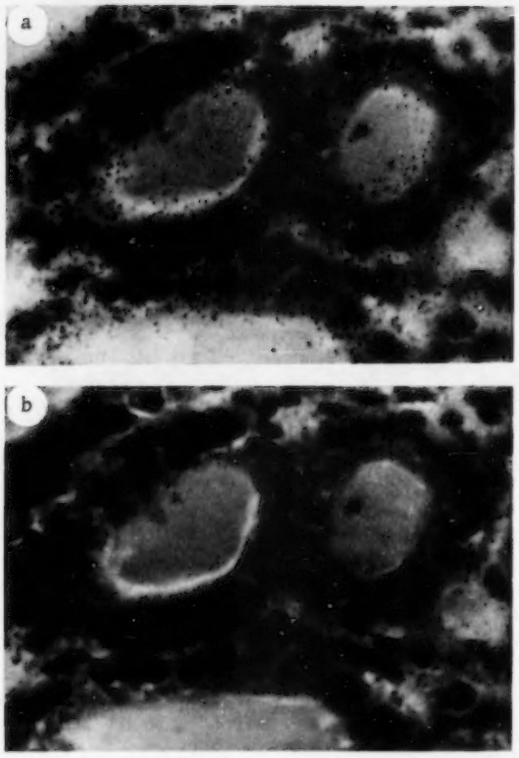


Fig. 2. Micro-autoradiograph of the follicles of the thyroid gland of a rat (the animal was killed 5 minutes after the intravenous injection of 5mC/kg of S^{35}-methionine). a) focused on the photographic emulsion. Concentration of the silver grains is observed over the follicular epithelium only; b) focused on the tissue; stained with hematoxylin-eosin; magnification 40 x 10.

At later periods an increase was observed in the number of follicles containing protein-bound radioactive iodine compounds. For instance, in rats sacrificed after 1 hour, practically all the follicles were radioactive. At this stage protein-bound radioactive iodine compounds also appeared in the cells of the follicular epithelium. The main accumulation of the iodized protein in the colloid was maintained, however, at whatever time the animals were sacrificed.

It was impossible to obtain micro-autoradiographs of the protein-bound S^{35}-methionine compounds 10 seconds after the intravenous injection of the labeled amino acid, even when an activity of 5-10 μC/g was used. Five minutes after injection of S^{35}-methionine, however, an obvious accumulation of sulfur-labeled protein was seen in the cells of the follicular epithelium. At this time hardly any radioactivity was present in the colloid (Fig. 2).

With lengthening of the interval between the injection of S^{35}-methionine and death of the animals, the concentration of labeled protein in the colloid rose, and after 24 hours it was considerably greater than its accumulation in the cells of the follicles. The greater accumulation of labeled protein in the colloid was also maintained at later stages after the injection of S^{35}-methionine.

The results obtained show that only 10 seconds after the intravenous injection of radioactive iodide, iodized protein was found in the colloid of the thyroid gland. We chose a time of 10 seconds because, after this time, the iodide would have passed from the place of injection through the remainder of the greater circulation and the lesser circulation and would have entered the tissue of the gland. It might have been expected that decapitation, leading to an instantaneous arresting of the circulation, would not have such a rapid paralyzing effect on the course of the metabolic processes in the thyroid gland. Extraction of the thyroid gland and trachea en bloc, and placing it in the cold fixing agent under our experimental conditions took about 10 seconds. A certain length of time was necessary also for diffusion of the cold fixative into the tissue. From the foregoing it follows that under these circumstances the usual course of the metabolic processes in the thyroid gland could continue for a further 15-20 seconds after the entry of the radioactive iodine into the gland. The obtaining of micro-autoradiographs of the thyroid gland at later times will evidently be fraught with great technical difficulties.

The experiment thus showed that for at least 15-20 seconds after the entry of the radioactive iodine into the thyroid gland, its protein-bound compounds were to be found exclusively in the colloid. The method of micro-autoradiography, like any other method, has certain limitations. For this reason it may be suggested that its sensitivity in this particular case was insufficient to show a low concentration of labeled protein in the cells of the follicles. By considering the density of the darkening of the photographic emulsion over the colloid, the background of the photographic emulsion, and length of the exposure, comparatively simple calculations will show what gradations of concentration of iodized protein between the colloid and the cells of the follicular epithelium would be necessary for it to be impossible to detect the presence of labeled protein in the cells. The calculations in our experiments showed that it would be possible to detect the presence of labeled protein in the cells in a concentration of 100-500 times less than in the colloid. It follows that 15-20 seconds after the entry of the iodine into the gland, not less than 99% of the newly formed iodized protein was present in the colloid.

On the basis of these findings, two hypotheses can be put forward regarding the site of iodization of the protein in the thyroid gland: iodization either takes place in the apical parts of the cells of the follicles of the thyroid gland at the moment that the protein molecule passes into the colloid, or iodization of the protein takes place in the cavity of the follicle in the colloid itself. There are reasons to believe that the uptake of labeled amino acids in the body mainly reflects the process of protein synthesis [6, 16]. The appearance of labeled protein in the follicle cells first may indicate that this is the place where thryroglobulin is synthesized, after which it passes into the cavity of the follicle.

The results obtained suggest that the processes of synthesis of the thyroglobulin molecule and its iodization in the thyroid gland occur in different places. Synthesis of the protein part takes place in the follicle cells, and iodization occurs either in the cavity of the follicle or at the border of the apical parts of the cells and the colloid at the moment when the thyroglobulin molecule passes into the cavity of the follicle.

For this reason the reports [7, 8] that the tissue of the thyroid gland has the power to accumulate radioactive iodine in a protein-bound form, the presence of which is coincidental with the time of appearance of follicular structures in the symplast are of interest. Hence it follows that colloid filling the cavity of the follicles may be regarded not as a depot for hormone but as a structural unit of the gland having a definite function, in which takes place one of the more important stages in hormone formation – the iodization of the protein.

The droplets of matter found in the follicle cells which resemble colloid in their staining properties are regarded by several workers as being fully identical with colloid and as products secreted into the cavity of the follicle. There are reports [6] that organic iodine compounds are present in these droplets of matter. We consider that the organically combined iodine found in the follicle cells must be regarded as a product of hydrolysis of colloid destined for secretion into the blood stream. This is suggested by the later appearance of protein compounds of iodine in the follicle cells than in the colloid.

In our investigations we never found labeled protein-bound iodine compounds in the cells of the symplast which were not organized into follicular structures. This fact may be evidence that, in normal conditions, they do not form part of the organ and do not participate in the formation of the hormone.

SUMMARY

Experiments were performed on rats. Using the micro – autoradiographic method the author determined the localization of the protein-combined I^{131} and S^{35}-methionine compounds in the thyroid gland 10 seconds, 5-20

minutes and 1, 4, 24 and 72 hours after the intravenous injection of the labeled preparations. The iodine-thyroglobulin with radioiodine inclusion appears first in the colloid. Inclusion of S^{35}-methionine into the thyroglobulin molecule occurs in the follicular epithelium cells. The data obtained demonstrate that thryoglobulin synthesis and its iodization in the thyroid gland occur at different sites. Thyroglobulin synthesis takes place in the follicular cells, while protein iodization — either in the cavity of the follicles or at the border of the apical parts of the cells and colloid. The above led to the convulsion that the thyroid gland colloid should be regarded not as a place of hormone storage but as a structural unit of the organ which participates in hormone formation.

LITERATURE CITED

[1] A. O. Voinar, The Biological Role of Trace Elements in Animals and Man. Moscow, 1953 [In Russian].

[2] Ya. V. Mamul', Zhurn. Obshchei Biol., 13, No. 4, 299-305 (1952).

[3] M. F. Merkulov, Scientific Papers of the N. I. Pirogov Second Moscow State Medical Institute, vol. 6, pp. 163-183, 1957 [In Russian].

[4] A. É. Sharpenak, E. A. Il'ina and O. N. Belashova, Biokhimiya, 11, No. 2, 133-138 (1946).

[5] J. B. Alpers, J. Robins and J. E. Rall, Endocrinology, 1955, v. 56, p. 110-119.

[6] L. F. Belanger, Anat. Record., 1956, v. 124, p. 555-571.

[7] G. A. Boyd and A. J. Williams, Proc. Soc. Exper Biol and Med., 1948, v. 69, p. 225-232.

[8] E. DeRobertis, Anat. Rec., 1941, v. 80, p. 219-231.

[9] J. Derrien, R. Michel, K. O. Pedersen and J. Roche, Biochem. Biophys. Acta, 1949, v.3, p.436-442.

[10] I. Doniach and S. P. Pelc, Brit. J. Radiol., 1950, v. 23, p. 134-192.

[11] I. Gersch and T. Casperson, Anat. Rec., 1940, v. 78, p. 303-320.

[12] A. Gorbman and H. M. Evans, Proc. Soc. Exper. Biol. and Med., 1941, v. 47, p. 103-106.

[13] J. G. Hamilton, M. H. Solev and K. B. Eichorn, Univ Calif., Berkeley, Publ. Pharmacol., 1940, v. 1, p. 339-367.

[14] H. Heath, C. Rimington and T. Glover et al., Biochem. J., 1953, v. 54, p. 606-611.

[15] C. P. Leblond and J. Gross, Endocrinology, 1948, v. 43, p. 306-322.

[16] C. P. Leblond, N. B. Everett and B. Simmons, Am. J. Anatomy, 1957, v. 101, p. 225-261.

[17] C. P. Leblond, W. L. Percival and J. Gross, Proc. Soc. Exper. Biol. Med., 1948, v. 67, p. 74-76.

[18] T. Mann and E. Leone, Biochem. J. 1953, v. 53, p. 140-148.

[19] L. E. Preuss and C. Harrison, Science, 1954, v. 120, N. 3106, p. 67-68.

[20] L. Roche, G. Delton, I. R. Michel and S. Lissitzky, Compt. rend. Soc. bio., 1950, v. 144, p. 1647-1649.

[21] H. Seymour, R. S. Wollman and S. Wodinsky, Endocrinology, 1956, v. 56, p. 9-20.

[22] B. Weiss, J. Biol. Chem., 1953, v. 205, p. 193-203.

THE EFFECT OF RIBONUCLEIC ACID AND ITS HYDROLYZATES ON THE UPTAKE OF GLYCINE-1-C^{14} BY PROTEINS OF NORMAL AND TUMOR TISSUES

I. B. Zbarskii and N. P. Karuzina

From the Biochemical Laboratory (Head — Prof. I. B. Zbarskii) of the P. A. Gertsen State Oncological Institute (Director — Prof. A. N. Novikov), Moscow

(Received February 6, 1959. Presented by Active Member AMN SSSR A. E. Braunshtein)

It is nowadays accepted that ribonucleic acid (RNA) plays a direct part in protein synthesis [6]. The stimulating effect of RNA or its hydrolyzates on the inclusion of radioactive amino acids in proteins has, however, been demonstrated in only a few investigations; for example, in damaged staphylococcal cells [7], in plants [10], and in sections of normal organs of the rat [3]. In the last of these investigations it was shown that the stimulation is inconstant in the case of cells of Ehrlich's ascitic cancer and practically absent in sections of rat sarcoma.

In the present research the effect of RNA on the incorporation of labeled glycine-1-C^{14} in protein was investigated mainly in minced normal organs and tumors of rats and also in sections of these tissues, and the relationship between this effect and the degree of hydrolysis of the RNA, the ages of the animals, and the time elapsing after transplantation of the tumor was also studied.

In connection with A. A. Khadzhiolov's findings that RNA hydrolyzates stimulate the respiration of yeasts [5], in some experiments the oxygen absorption was simultaneously estimated.

EXPERIMENTAL METHOD

The investigation was carried out on the liver, kidneys, spleen, brain, pancreas, and thymus, and also on transplanted tumors (cholangioma and M-1 sarcoma) of rats. The tissue was minced finely with scissors in the cold for $1\frac{1}{2}$-2 minutes.

RNA was used both in the form of neutralized hydrolyzates obtained by hydrolysis with 2.5N KOH at 37° for various periods of time, and also in the form of an unhydrolyzed preparation of sodium ribonucleate (from yeast). The preparation did not give the biuret reaction for protein and did not contain inorganic phosphorus. When dried over P_2O_5 in a vacuum exsiccator, the preparation contained 13.8% nitrogen and 7.2% phosphorus (ratio N/P = 1.97).

Experimental samples (usual volume 2 ml) consisted of 1.6 ml of Robinson's medium [9] of 0.1% glucose; 0.3 ml of RNA solution or hydrolyzate (final concentration 0.05%); an average of 0.1 ml of a solution of glycine-1-C^{14} (100 000 impulses per minute) and 150-250 mg of sections or mince in the case of normal tissues or 300-350 mg (in view of the higher water content) in the case of tumor tissue.

Incubation was carried out in Warburg flasks for 50 minutes in an atmosphere of O_2 at 38°.

Controls were set up without RNA and containing RNA or its hydrolyzates but not incubated. After incubation, trichloroacetic acid was added to give a final concentration of 5 %. Trichloroacetic acid was added to the unincubated control samples immediately after the sections or mince had been placed in the medium containing

TABLE 2

Duration of hydrolysis (in hours)	Number of experiments	Stimulation (in %)
0	4	+36.3
3	2	+12.5
6	6	+70.6
9	1	+34.0
12	5	+30.7
18	1	+77.0
24	1	+66.0
48	2	+29.4
Average	22	+44.6±8.5

Stimulation of Uptake of Radioactivity of Glycine-1-C^{14} into the Proteins of Minced Rats' Pancreas from Animals of Different Ages, Depending on the Duration of Hydrolysis of RNA

TABLE 1

The Influence of RNA Hydrolyzates on the Uptake of Glycine-1-C^{14} into the Proteins of Sections of Normal Organs of Rats

$\left(\frac{\text{imp/min/g protein}}{\text{imp/min/ml medium}}\right)$

Organ	Number of experiments	With RNA	Without RNA	Change (in %)
Liver	10	0.63	0.59	+8.8±6.2
Kidney	10	1.43	1.28	+13.0±15.0
Spleen	7	1.66	1.90	−8.2±7.0

all the components of the test sample. The residue was ground and washed with 5% trichloroacetic acid, the nucleic acids were extracted with 5% trichloroacetic acid at 90° for 20 minutes, and the lipids with a mixture of alcohol and ether; the usual method was used to determine the radioactivity of 10 mg of protein on aluminum disks [2] by means of an end-type counter on a B apparatus.

The radioactivity was expressed in relative values: $\frac{\text{impulses per minute per g protein}}{\text{impulses per minute per ml medium}}$. The numbers of impulses in the control samples (not incubated) was close to the background level. As an example, in the experiment with minced brain, the experimental sample gave 584 impulses per minute, the control 27 and the background level 25; in the experiment with minced pancreas the corresponding values were 2500 impulses per minute with 32 for the control and 23 for the background.

The oxygen absorption was estimated by Warburg's standard method [4] and expressed as μ liters O_2 per 100 mg raw tissue during the time of the experiment.

EXPERIMENTAL RESULTS

In the majority of experiments with sections of liver, kidneys, and spleen from rats (weighing from 40 to 170 g) in the presence of RNA hydrolyzates (duration of hydrolysis 19, 24 and 48 hours) the incorporation of the radioactivity of the glycine into the proteins was slightly higher than that in the absence of RNA, although the difference was not statistically significant (Table 1).

The experiments on the pancreas, as an organ characterized by a high intensity of protein synthesis, were carried on further. Since we were unable to obtain satisfactory sections of rats' pancreas, these and the subsequent experiments, including those on other organs, were carried out on minced preparations.

In the experiments with minced rats' pancreas, both RNA and its hydrolyzates definitely stimulated the uptake of the radioactivity of the glycine into the protein. This effect, however, did not depend on the duration of hydrolysis of the RNA, as in the experiments on sections of liver, kidney, and spleen described above (Table 2).

For the experiments on minced preparations, the pancreas and brain were taken from rats of different ages. Stimulation was observed in rats of various weights, but the younger the animal, the more marked the stimulation (Table 3). Similar results were obtained in minced brain.

In the experiments on minced preparations of other organs of rats, RNA and its hydrolyzates also had a stimulating action on the uptake of the radioactivity of glycine-1-C^{14} into the proteins. Table 4 shows the results of these experiments treated statistically.

In the experiments on tumors we used M-1 sarcoma and a cholangioma — an alveolar carcinoma of the liver, originally induced by 2-acetylaminofluorene, a slowly growing tumor with a small number of necrotic areas that appeared only in the later stages of its growth.

TABLE 3

The effect of RNA Hydrolyzate (Hydrolysis for 6 Hours) on the Uptake of Radioactivity of Glycine-1-C^{14} into the Proteins of Minced Pancreas of Rats of Different Ages (imp/min/g protein) / (imp/min/ml medium)

No. of rats	Weight of rat (in g)	With RNA	Without RNA	Change (in %)
5	16—18	5.71	2.92	+95.0
3	17—25	7.29	3.34	+118.0
3	26—31	6.94	4.02	+72.7
9	28—34	4.32	3.54	+22.0
6	49—67	5.99	5.65	+6.0

TABLE 4

The effect of RNA and its Hydrolyzates on the Uptake of Glycine-1-C^{14} into the Proteins of Minced Normal Organs of the Rat (imp/min/g protein) / (imp/min/ml medium

Organ	Number of experiments	With RNA	Without RNA	Change (in %)
Pancreas	22	6.07	4.44	+44.2±8.5
Liver	4	1.60	1.09	+45.1±8.5
Kidney	4	2.00	1.50	+40.1±16.9
Spleen	4	1.55	1.08	+61.3±30.1
Brain	20	0.76	0.48	+85.0±18.2
Thymus	5	2.45	2.17	+13.2±11.0

In the case of minced tumor preparations, the effect of RNA and its hydrolyzates on the uptake of the radioactivity of the glycine into the proteins was slight and occasionally even amounted to depression of the uptake. For instance, in 6 experiments on minced sarcoma M-1, the uptake in samples without RNA averaged 0.65, and with the addition of RNA the uptake was 0.56. The average change was 7.1 ± 17.7%. The duration of hydrolysis of RNA, and also the length of time after transplantation of the tumor did not infulence the observed effect.

The results of the next experiments, in which the oxygen demand was determined in addition to the uptake of radioactivity in the same minced preparations, are shown in Table 5 for sarcoma M-1 and in Table 6 for cholangioma. In the case of the sarcoma M-1, as in the previous experiments, RNA produced no statistically significant changes either in respiration or in the uptake of radioactivity. The time elapsing after the transplantation of the tumor was also without effect. In the case of the cholangioma, in early stages of growth (15-22 days after transplantation) some degree of stimulation of incorporation of the amino acid took place, but this was shown less clearly than in the normal organs. At later stages of growth (30th-48th day after transplantation) the uptake of radioactivity in the experimental and control (no RNA added) samples was practically identical. RNA behaved in the same way towards the respiration of the minced cholangioma. The effect on respiration was shown very feebly, but comparison of the respiration and the uptake of radioactivity in the same experiments indicates a slight positive correlation between those two metabolic indices.

TABLE 5

Uptake of Glycine-1-C^{14} into Proteins $\left(\frac{\text{imp/min/g/protein}}{\text{imp/min/ml medium}}\right)$, and Respiration ($\mu$ liters O_2/100 mg fresh tissue during time of experiment) of Minced Transplanted Sarcoma M-1 of a Rat

No. of experiments	Respiration			Uptake		
	with RNA	without RNA	change (%)	with RNA	without RNA	change (%)
6	46.0	43.7	+9.6±5.5	0.86	0.81	+15.3±15.8

TABLE 6

Incorporation of Glycine-1-C^{14} into Proteins $\left(\frac{\text{imp/min/g/protein}}{\text{imp/min/ml medium}}\right)$ and Respiration (μ liters O_2/100 mg fresh tissue during time of experiment) of Minced Transplanted Cholangioma of a Rat at Different Stages of Growth of the Tumor

Time after transplantation	Respiration				Incorporation			
	Number of experiments	with RNA	without RNA	change (%)	Number of experiments	with RNA	without RNA	change (%)
From 15 to 22 days	8	18.7	16.6	+13.8±5.52	8	1.78	1.49	+14.8±3.84
From 30 to 48 days	4	11.4	13.6	−18.1±9.52	6	0.53	0.56	−1.3±11.7

The action of RNA hydrolyzates on the uptake of the radioactivity of the glycine into the proteins of the sections and minced preparations of normal organs and transplanted tumors did not depend on the duration of hydrolysis (from 0 to 48 hours). It was more marked in rats weighing less than 25-30 g than in older animals.

In the experiments on the cholangioma, the effect of RNA on the resoipration of the minced preparations was in general similar to its effect on the incorporation of glycine into the proteins. In individual experiments a correlation was observed between these two values.

The results obtained do not permit any conclusion to be drawn concerning the mechanism of stimulation of incorporation of glycine-1-C^{14} nor the active principle responsible for this effect. It is possible that this principle is not nucleotide in nature but is to some extent analogous to the factor described by Gale and Folkes [8]. There is also the possibility that the action of RNA on glycine uptake is due to the influence on respiration, with a corresponding increase in the energy metabolism [5]. Finally, it may be postulated that the observed effect depends on the stimulation of the synthesis of RNA itself [1, 3].

The absence of any effect of RNA on minced preparation of tumors indicates that their metabolism is of a different character. It is apparent that RNA has no effect on minced preparations of tumors in late stages, when partially necrotic, but it does affect recently transplanted, intensively growing tumors. In this respect a characteristic difference is seen between the early and late stages of cholangioma — a slowly growing tumor in which necrosis occurs only in late stages of growth — and the absence of effect on the various stages of growth of sarcoma M-1 — a tumor in which diffuse necrosis develops even in the early stages.

The fact that the effect is well marked in the minced preparation and is almost absent in the normal tissue slices evidently implies that the action of RNA is best shown when the cells are partially destroyed. The marked effect obtained in previous work on slices [3] probably depended on the fact that the slices were damaged more than in the present investigation, when they were prepared very carefully.

SUMMARY

Ribonucleic acid and its hydrolyzates exert a stimulating effect on the incorporation of glycine-1-C^{14} into the proteins of minced normal organs (pancreas, liver, kidneys, spleen, brain, and thymus) of rats and have almost no effect on the incorporation of glycine radioactivity into the proteins of minced sarcoma M-1 and transplanted cholangioma at late stages of its growth. At early stages of cholangioma growth some stimulation of radioactivity incorporation is noted. The effect of the stimulation is independent of the length of RNA hydrolysis.

LITERATURE CITED

[1] A. K. Belousov, Biokhimiya, 20, 4, 495 (1955).

[2] L. B. Zbarskii and K. A. Perevoshchilova, Doklady Akad. Nauk SSSR, 107, No. 2, 285 (1956).

[3] K. A. Perevoshchikova and I. B. Zbarskii, Doklady Akad. Nauk SSSR, 114, No. 1, 150 (1957).•

[4] V. V. Umbreit et al., Manometric Methods of Study of Tissue Metabolism. Moscow, 1951 [In Russian].

[5] A. A. Khadzhiolov, Izvest. Otdel. Biol. Med. Nauk Bolgarskoi AN, No. 1, 123 (1957).

[6] J. Brachet, Biochemical Cytology, New York, 1957.

[7] E. F. Gale and J. P. Folkes, Biochem. J., 1953, v. 55, N. 1, p. XI; N. 5, p. 721.

[8] E. F. Gale and J. P. Folkes, Nature, 1958, v. 182, p. 94-97; Biochem. J., 1958, v. 69, p. 611.

[9] L. R. Robinson, Biochem J., 1949, v. 45, p. 68-74.

[10] G. C. Webster and M. P. Johnson, J. Biol. Chem., 1955, v. 217, p. 641.

• Original Russian pagination. See C. B. Translation.

CHANGES IN THE AMMONIA AND GLUTAMINE CONTENT OF THE BRAIN IN RATS AFTER ADMINISTRATION OF SODIUM BROMIDE AND CAFFEINE AND IN CARBON TETRACHLORIDE POISONING

B. G. Gordon

From the Laboratory of Biochemistry of the Nervous System (Head – Corresponding Member AMN SSSR G. E. Vladimirov) of the I. P. Pavlov Institute of Physiology (Director – the late K. M. Bykov) of the AN SSSR, Leningrad

(Received February 28, 1959. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In previous research [1, 2] carried out jointly with N. V. Veselkin, we found an increase in the content of ammonia and glutamine in the brain of animals during disturbances of the liver activity caused by surgical interference and by poisoning of the animal with CCl_4.

In the present investigation we studied the effect of sodium bromide and caffeine on the content of ammonia and glutamine in the brain of white rats during CCl_4 poisoning.

EXPERIMENTAL METHOD

We know from the work of the Pavlov school that, depending on the state of the nervous system and the type of higher nervous activity of an animal or man, the dose of sodium bromide necessary to produce a beneficial effect varies considerably. In our investigation we therefore used both high and low doses of sodium bromide: either a dose of 20-50 mg/100 g body weight subcutaneously 2-3 times a day for 2-5 days (large doses) or 1-5 mg/100 g body weight once a day for 24-30 days (small doses); Caffeine (sodium benzoate) was injected subcutaneously in a dose of 3-5 mg/100 g body weight once or twice a day for 2-5 days.

CCl_4 was injected intraperitoneally as a single dose of 0.1-0.2 mg/100 g body weight in 1 ml of mineral oil. The animals were sacrificed by immersion in liquid oxygen. The frozen brain was extracted and the content of ammonia and glutamine determined. Ammonia was estimated by vacuum distillation of a trichloroacetic centrifugate of the brain (in a Parnas apparatus); the amide nitrogen content of the glutamine was investigated in a separate sample of the same centrifugate by the increase in ammonia after acid hydrolysis for 11 minutes on a boiling water bath.

EXPERIMENTAL RESULTS

No increase in ammonia and glutamine was found in rats after administration of caffeine, for our results were the same as those which we obtained in normal rats in a state of increased physiological activity (Fig. 1, a). In these conditions the rats appeared healthy, with the exception that they were more restless and timid.

After the prolonged administration of small doses of sodium bromide to the rats, as might have been expected, we found no increase in the content of ammonia and glutamine in the brain (Fig. 1, b). Outwardly, the rats appeared perfectly healthy, very alert and mobile. After administration of large doses, however, the general condition and behavior of the rats was greatly modified. During the first few days of the injections the rats slept soundly at unusual times of day, and signs of bromide poisoning became apparent and developed in intensity. Under these circumstances the animals appeared very sluggish, their fur became ruffled and their skin pale or cyanotic. They often did not react even when grasped with forceps during freezing.

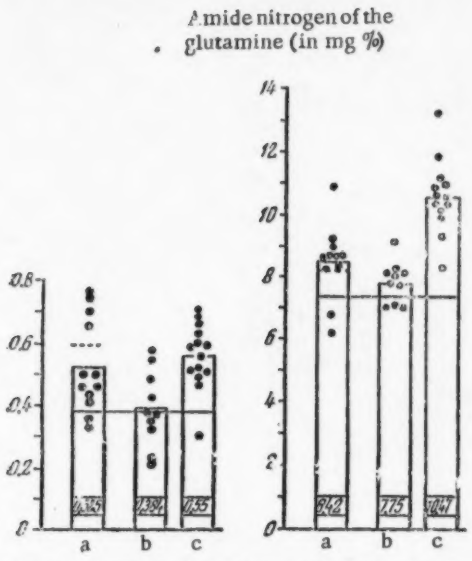


Fig. 1. Content of ammonia and glutamine in the brain of rats. a) After injection of caffeine; b) of sodium bromide (small doses); c) sodium bromide (large doses). Continuous line — a normal animal in a state of physiological rest. Broken line — normal animal in a state of physiological activity. Along the ordinate axis — NH_3 as nitrogen (in mg %).

The results obtained from analyses of the brain showed that the content of ammonia and glutamine in the brain of these rats was greatly increased, by as much as 45% in the case of ammonia and 43% for glutamine (Fig. 1, c).

From 1 to 2 hours after the injection of CCl_4, in spite of the very poor general condition of the rats, the content of ammonia and glutamine in their brain was within normal limits (Fig. 2, A). These results were in agreement with those in the literature [9], showing that obvious histological changes begin to appear in the liver 4 hours after exposure to the action of CCl_4.

Twenty four hours after a single injection of CCl_4 central necrosis developed in the liver, affecting from a quarter to a half of each lobule [10], and as a results of this the ammonia content of the liver was increased [2]. Evidently this was also the cause of the increase in the content of ammonia and glutamine in the brain tissue also, as we found in our previous work. According to the results of our present investigation, after 24 hours the content of ammonia in the brain tissue had increased on the average by 87% and that of glutamine by 83% (Fig. 2, B).

These findings confirm that the presence of extensive damage to the liver tissue may result, in fact, in the appearance of excessive amounts of ammonia in the brain.

If CCl_4 was injected 2-5 days after administration of caffeine, the general condition of the rats both in the hours immediately after injection and after 24 hours was rather better than in control animals (given CCl_4 only), but as a rule their characteristic hypermobility was absent. If the results obtained were compared with the normal values for a resting state, the increase in the ammonia content was +61%. That an increase in the ammonia content of the brain did in fact result from the harmful effect of CCl_4 on the liver was supported by a simultaneous absolute increase in the glutamine content of +67% compared to the resting state or of +49% compared to a state of physiological activity (see Fig. 2, a).

If rats were injected with CCl_4 after receiving small doses of sodium bromide for a long time or large doses for a short time, the increase in the content of ammonia and glutamine in the brain was very high, namely +74% and +80% respectively with the small doses and +66% and +77% with the large doses (Fig. 2, b and c). The general behavior of the rats during the 36 hours after injection of CCl_4, especially when large doses of sodium bromide had been given, was very seriously affected, and they appeared much worse than those animals which received CCl_4 alone in parallel experiments. If all three groups of rats are compared under these circumstances (receiving CCl_4 alone or CCl_4 after caffeine or sodium bromide), the impression is created that caffeine supported the general condition of the rats but sodium bromide caused it to deteriorate considerably. If, however, attention is directed towards the rate of survival of the rats, it is apparent that among the rats which received CCl_4, after caffeine, cases occurred in which the animals died in spite of their outwardly better condition, whereas after receiving even large doses of sodium bromide and CCl_4, in spite of their serious condition, the animals did not die. This may be due to the lowering, by the action of sodium bromide, of the sensitivity of the central nervous system to the toxic agents (CCl_4 and ammonia). A similar phenomenon has been observed by other authors [4, 5]. In a state of prolonged drug-induced sleep, extremely high contents of ammonia and glutamine were found in the brain of the animals as the result of the disturbance of the urea-forming function of the liver under these conditions, although the animals did not die [5]. In our experiments, therefore, the administration of caffeine, and especially of sodium bromide, during poisoning with CCl_4 had no appreciable effect on the content of ammonia and glutamine in the brain. At the same time we consider of interest the fact that the content of ammonia and glutamine in the brain was increased when the rats were poisoned with large doses of sodium bromide. The

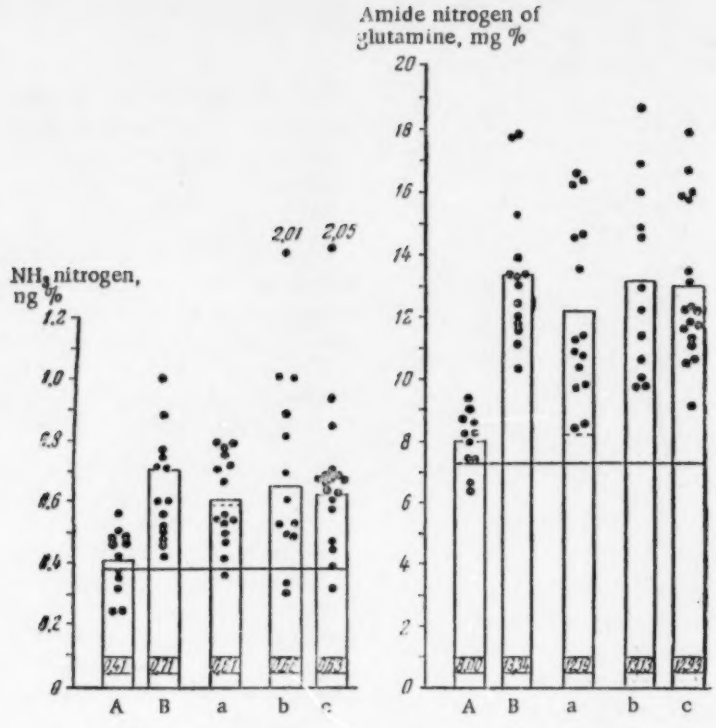


Fig. 2. Content of ammonia and glutamine in the brain of rats. A) 1-2 hours after injection of CCl_4 and B) 24 hours after injection of CCl_4; a) after injection of caffeine + CCl_4; b) of sodium bromide + CCl_4 (small doses); is c) of sodium bromide + CCl_4 (large doses). In columns b and c the means values of the NH_3 nitrogen were calculated without taking into consideration the results of two experiments in which a very high ammonia content was obtained (2.01 and 2.05 mg%).

manifestations of bromism are well known clinically – in persons receiving an overdose of bromide the speech is disturbed and impairment of consciousness, delirium and cachexia, etc., develop. This condition is reminiscent of the state of coma arising in hepatic failure, when low values are established for the oxygen consumption of the brain and for the absorption of ammonia by the brain from the blood (the blood of these patients often contains increased concentrations of ammonia [6, 7, 8]). A fall in the oxygen demand in these cases precedes the appearance of neurological signs. A lowering of the oxygen demand of the brain and liver tissue is also found in prolonged drug-induced sleep, when, as has previously been mentioned, the urea-forming function of the liver is disturbed and, in connection with this, the content of ammonia and glutamine in the brain is increased [3, 5].

The lowered oxygen consumption of the brain in coma is explained by some authors [6, 7, 8] by blocking of the formation of intermediate metabolites of the three-carbon acid oxidation cycle by ammonia, which also disturbs the normal course of the oxidative processes in the brain and leads to the development of a number of neurological disorders.

The sharp increase which we found in the content of glutamine in the brain during disturbance of liver function and after administration of large doses of sodium bromide may be indirect evidence in favor of this point of view, and may also help towards an understanding of the mechanism of the toxic action of large doses of bromides.

SUMMARY

The ammonia and glutamine content in the brain of rats is not increased by the action of caffeine and low doses of sodium bromide. Conversely, the animals' behavior and the content of ammonia and glutamine in the brain rises under the effect of large doses of sodium bromide.

Increase of the ammonia and glutamine content in the brain occurring in carbon tetrachloride poisoning does not drop noticeably under the effect of caffeine or sodium bromide. Investigations of rats' brain at different intervals after CCl_4 administration (in 1-2 hours and in 24 hours) show that the increase of the ammonia and glutamine content in the brain occurs only when marked injury of the liver tissue takes place, i.e., in 24 hours, notwithstanding the fact that the general condition of rats is particularly grave in 1-2 hours. This confirms the supposition that disturbances of the central nervous system occuring in diseases of the liver may be also due to the action of ammonia.

LITERATURE CITED

[1] N. V. Veselkin and B. G. Gordon, Byull. Éksptl. Biol. i Med., 47, No. 3, 34 (1959).•

[2] É. A. Gordienko, Proceedings of a Conference of Junior Scientific Workers of the Institute of Hygiene of Work and Occupational Diseases, p. 26, Moscow, 1957 [In Russian].

[3] G. G. Gur'yanova, Changes in the Respiration of the Brain, Heart and Liver and in the Adenosinetriphosphate Content of the Brain during Prolonged Drug-Induced Sleep. Author's summary of dissertation, Leningrad, 1958 [In Russian].

[4] K. S. Kosyakov, Farmakol. i Toksikol., No. 4, 17 (1956).

[5] É. É. Martinson and L. Ya. Tyakhepyl'd, The Biochemistry of the Nervous System, p. 176. Kiev, 1957 [In Russian].

[6] S. P. Bessman and A. N. Bessman, Clin. Invest., 1955, v. 34, p. 622.

[7] S. P. Bessman, J. F. Fazekas and A. N. Bessman, Proc. Soc. Exper. Biol. and Med., 1954, v. 85, p. 66.

[8] J. F. Fazekas, H. E. Ticktin and W. R. Ehrmantraut, et al., Am. J. Med., 1956, v. 21, p. 843.

[9] T. P. Bharadwaj and P. P. Nair, Indian. J. Med. Sc., 1956, v. 10, p. 651.

[10] J. Hoffman, M. B. Himes and S. Lapan et al., Arch. Path., 1955, v. 59, p. 429.

• Original Russian pagination. See C. B. Translation.

THE BIOLOGICAL ROLE OF URANIUM IN THE MAMMALIAN BODY

D. P. Kladienko

From the Department of Histology (Head – Docent I. A. Shevchuk)
of the Chernovtsy Medical Institute (Director – Docent M. M. Kovalev)

(Received May 25, 1959. Presented by Active Member AMN SSSR
V. N. Chernigovskii)

Uranium is known to be present in nearly all human tissues and organs, and according to Hoffmann[5], the thyroid gland is the depot for uranium compounds.

In a previous communication [2] we showed that the action of uranium nitrate in our experiments could be compared with the action of certain thyroid gland inhibitors: thiourea, thiouracil, etc., which block the follicular cells of the thyroid gland and thereby inhibit the hormone-producing function. Hence it may be concluded that this element bears some relationship to the function of the thyroid gland. In order to study this problem, we undertook a series of experimental investigations on white rats and rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 67 experimental and 20 control rats of both sexes, aged $2^1/_2$-3 months; 40 rats received 0.05 mg of uranium (uranyl) nitrate per 100 g body weight in milk daily for 7 months, and the remaining 27 animals received 0.01 mg/100 g body weight in the form of subcutaneous injections. Each rat received on the average of 10 mg of uranium in milk or 2 mg by subcutaneous injection. The rats were weighed every 2 weeks. The average initial weight of the rats was 122 g, and the average final weight 200 g. After 7 months the rats were sacrificed for the purpose of histological examination of their organs – the thyroid gland, the hypophysis, the adrenals and the spleen.

Fig. 1. The thyroid gland of a rabbit after receiving uranium nitrate for 1 month. The colloid is thick and highly vacuolated. Many interfollicular cells. Ocular $10\times$, objective $40\times$.

Experiments were also performed on rabbits. Ten rabbits were used initially for an investigation of the normal functional state of the thyroid gland by means of administration of radioactive iodine (I^{131}) to the animals in a dose of 1.5 μC to each animal. One month later, the same rabbits were given subcutaneous injections of 0.1 mg uranyl per kg body weight in 1 ml distilled water daily for 30 days. At the end of the course of injections the rabbits were again given the same dose of I^{131} as before (1.5 μC) in order to ascertain any possible changes in the functional activity of the gland. Six rabbits were used as controls and received no treatment.

The activity of the thyroid gland in absorption of I^{131} was measured as γ-irradiation by means of a pickup and a

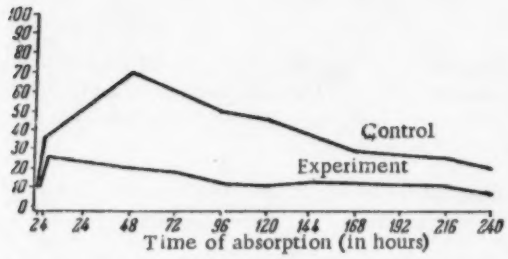


Fig. 2. 10-day absorption curves of radioactive iodine by the thyroid gland of normal rabbits (upper curve) and of the same rabbits after receiving uranium nitrate (lower curve). Vertical axis — absorption of I^{131} as % of total activity of dose injected.

"B" apparatus 2, 4 and 24 hours after administration of the I^{131}, and thereafter once a day for 10 days. The activity of absorption of I^{131} by the gland was expressed as a percentage of the total activity of the dose administered.

EXPERIMENTAL RESULTS

No difference was found between the growth of the experimental and control rats. The average weight of the control rats at the end of the experiment was 190 g, and the average weight of the experimental rats was 200 g. In contrast to the use of large doses (2.5%) of uranyl in the food [3], the above doses evidently had no marked effect on growth. At the same time it must be pointed out that a considerable number of the experimental rats died (32 and 37%). The mortality was especially high in the last 3 months of the experiment. At necropsy the majority of the animals showed foci of suppuration in the lungs, and in some animals there were lesions of the cervical lymphatic glands. The glands were greatly enlarged and pus was present inside them.

No death of the control animals was observed. From the above it may be deduced that the cause of death of the animals was slow poisoning with uranyl.

Significant histological changes were found in the thyroid gland of the experimental rats. In the majority of cases the follicles were small, the epithelium cubical, and the colloid thick and highly vacuolated. The number of cells of the interfollicular epithelium was far in excess of normal. In the adrenals a considerable thickening of the cortex was observed without visible destruction of the cortical and medullary substance. No appreciable abnormalities were found in the hypophysis and spleen.

The rabbits were incomparably more sensitive to uranyl than the rats. According to Haven and Hodge [4], the mean lethal dose for subcutaneous injection to rabbits is 0.7 mg/kg body weight, and accordingly we used one seventh of the lethal dose. The subcutaneous injection of 0.1 mg/kg body weight of uranyl daily for 30 days caused no outwardly visible pathological signs, but on the 20th day of the experiment a considerable quantity of albumin appeared in the urine of the rabbits. If it is remembered that in the course of the month each rabbit received 6 mg of uranyl, the toxicity of this element will be obvious.

Histological examination showed that the number of basophilic cells in the hypophysis was increased. The sinuses of the spleen were engorged with blood and dilated. The morphological changes in the thyroid gland were the most demonstrative: the follicles were small and the epithelium cubical. Occasionally large follicles were encountered with Sanderson's cushions* The colloid was thick and highly vacuolated (Fig. 1). A large number of interfollicular cells was observed.

The results of the investigation of absorption of I^{131} by the thyroid gland are shown in Fig. 2. The upper curve indicates the mean absorptive power of the gland in normal rabbits, the lower — the mean absorptive power of the gland in the same rabbits after treatment with uranyl. The variations between the values obtained in individual rabbits were insignificant. Comparison of these curves showed convincingly that uranyl, even in very small doses, has a depressing effect on the function of the thyroid gland. The peak I^{131} absorption in the experimental series is far lower than that in the controls, and is displaced to the left. This suggests a significant fall in the absorptive power of the gland. Whereas the maximum absorption of I^{131} in the control series shows over 70% of the total activity of the dose injected, the maximum absorption in the experimental series is about 25%, i.e., slightly more than one third. It is also interesting that the lower curve is of a character which greatly resembles the curve of myxedema as shown in the paper by N. A. Gabelova [1].

It may thus be concluded from the foregoing that uranyl, when given to rabbits even in small doses, causes great changes in both the structure and the function of the thyroid gland or, in other words, uranyl behaves like certain strongly acting inhibitors towards the thyroid gland.

* Name not verified.

SUMMARY

Uranium nitrate injected subcutaneously into rabbits in the dose of 0.1 mg/kg of body weight for a period of 30 days causes a considerable depression of the ability of the thyroid gland to include radioiodine administered to the animal. The gland is enlarged and the changes in the parenchyma histologically resemble those seen in goiter.

A similar histological picture was observed in rats; the latter are much less sensitive to uranium salts than rabbits. Thus, uranium nitrate (uranyl) is a natural powerful inhibitor of the thyroid gland, and may be one of its natural goiterogenic factors.

LITERATURE CITED

[1] N. A. Gabelova, The Use of Radioactive Isotopes in Medicine, pp. 74-78. Moscow, 1953 [In Russian].

[2] D. P. Kladienko and R. A. Nadopta, Doklady Akad. Nauk SSSR, 115, 6, 1217-1219 (1957).•

[3] E. A. Maynard and H. S. Hodge, The Pharmacology and Toxicology of Uranium Compounds. Moscow, 1951 [Russian translation].

[4] F. L. Haven and H. S. Hodge, The Pharmacology and Toxicology of Uranium Compounds, vol. 2, pp. 22-35. Moscow, 1951 [Russian translation].

[5] J. Hoffmann, Biochemische Ztschr., 1943, Bd. 313, H. 5-6.

• Original Russian pagination. See C. B. Translation.

PHARMACOLOGY

THE EFFECT OF DIBASOL* ON THE AUDITORY ADAPTATION

A. P. Velitskii

From the Department of Pharmacology, Pharmacy and Pharmacognosy (Head – Honored Scientist Prof. N. V. Lazarev) and the Department of Otolaryngology (Head – Honored Scientist Prof. K. L. Khilov) of the S. M. Kirov Order of Lenin Military Medical Academy, Leningrad

(Received September 15, 1958. Presented by the late Academician K. M. Bykov)

The majority of research workers now consider that adaptive changes in the sensation of hearing are mainly regulated by processes taking place in the central nervous system. According to G. V. Gershuni [2], O. L. Nemtsova [6], and G. I. Grinberg and F. Kh. Fridlyand [3], auditory adaptation is due to the development of an inhibitory process in the cortical end of the auditory analyzer.

In view of reports in the literature [1, 5, 7 and others] that dibasol has the power of abolishing certain forms of inhibition in the central and peripheral nervous system and of preventing its development, in the present investigation we set out to study the effect of dibasol on the character of the adaptive changes in the sensation of hearing which developed in persons with normal hearing and with various lesions of the auditory analyzer under the influence of excessive noise.

EXPERIMENTAL METHOD AND RESULTS

We carried out 2 series of observations: the first series on 13 healthy young persons with normal hearing and the second on 20 persons with normal hearing and 39 persons with various forms of deafness. The investigations were made in the acoustics laboratory of the Leningrad Research Institute of Diseases of the Ear, Throat, Nose, and Speech, using an Atlas audiometer. In the first series of observations the method of investigation was as follows. The threshold of audibility of the subjects was measured for frequencies of 512, 2048 and 4096 cps monaurally, in the right ear; each then took a dose of 0.12 g of powdered glucose. After a rest of half an hour; an excessive sound of frequency 2048 c/sec and intensity 100 db above threshold level of audition was applied to the subject's right ear through an air telephone for 4 minutes. Immediately after the cessation of the sound stimulus, the acuity of hearing a sound of 512 cycles/sec was determined. Measurements of the thresholds of audibility of this sound were repeated at intervals of 30 seconds until the threshold value had returned to its initial level. After repetition of the excessive noise, just as at the beginning of the observations the acuity of hearing and the time taken for reversion of the adaptation to a frequency of 2048/sec, and then for one of 4096/sec, were determined by the method described above.

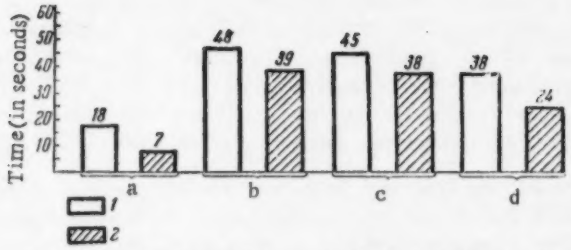
At the conclusion of these control observations with glucose, after 4-5 days the action of dibasol was tested on the same subjects. Observations were carried out by the same method, but instead of glucose the subject received a mixture of 0.1 g glucose with 0.01 or 0.005 g dibasol. The subjects were unaware of the composition of the powder.

The results obtained are shown in the table, which gives the values of the maximum loss of the sensation of hearing in decibels and the time of reversal of adaptation in seconds. Under the influence of dibasol in doses

* Dibasol is a vasodilator drug – 2-benzyl-benzimidazol hydrochloride (Translator's note).

The Action of Dibasol on the Adaptive Changes in the Sensation of Hearing

Investigation		Control						Experiments					
		maximum loss of sensation of hearing in db			time of reversal of adaptation (in seconds)			maximum loss of sensation of hearing			time of reversal of adaptation (in seconds)		
		frequency (beats/sec)											
name	age (in years)	512	2048	4096	512	2048	4096	512	2048	4096	512	2048	4096
Dibasol in a dose of 0.005 g													
A-n	19	—5	5	5	420	240	240	—5	5	10	300	120	300
K-v	20	0	10	15	30	270	330	0	7	15	30	120	180
K-v	19	0	10	5	30	390	270	—10	5	5	60	150	60
A-v	19	—5	5	10	90	300	660	—5	10	15	90	210	570
N-n	19	—5	5	10	180	240	240	—5	5	10	240	120	270
Z-v	22	5	10	5	120	210	180	5	5	5	60	120	150
V-n	19	10	5	10	240	180	330	10	5	10	150	90	150
Dibasol in a dose of 0.01 g													
M-v	23	10	10	5	240	210	150	2	10	5	150	120	90
A-v	22	15	15	5	240	270	90	5	5	0	90	90	30
M-n	21	5	10	15	60	210	240	0	5	5	30	90	120
B-v	22	5	5	10	120	330	180	5	5	5	60	360	60
K-n	19	15	5	10	600	60	60	5	0	10	90	30	120
I-v	21	—5	10	15	60	210	270	0	10	5	30	60	60



Changes in the time of reversal of adaptation in healthy subjects and in persons suffering from auditory neuritis and stages I, II and III otosclerosis, after taking glucose (0.1g) and a mixture of dibasol (0.01 g) with glucose (0.1 g). 1) Action of glucose; 2) action of a mixture of dibasol and glucose; a) Healthy subjects; b) auditory neuritis; c) stage III otosclerosis; d) stages I and II otosclerosis.

of 5 and 10 mg, the value of the maximum loss of hearing of the frequencies tested, and of the time of reversal of adaptation, were lowered by comparison with the controls in the majority of cases. The thresholds of audibility in both control and experimental series were changed not only to the stimulating tone (2048/sec), but also to tones of mixed frequency (512 and 4096/sec). Furthermore, with an increase in the dose of dibasol, the time for reversal of adaptation was correspondingly shortened for all the test frequencies.

The results of these observations also showed that the value of the maximum loss of the sensation of hearing and of the time of reversal of adaptation were, in the majority of cases, directly proportional to each other. On account of the laboriousness of the method which we have described, in the second series of observations we accordingly confined ourselves to an investigation of the time of reversal of adaptation alone.

The second series of observations was made on 20 healthy persons aged from 20 to 30 years, with normal hearing, and on 39 patients. The latter group was composed as follows: 14 persons with diseases of the auditory nerves, 14 with stage III otosclerosis and 11 with stage I or partly stage II otosclerosis. By age the patients were subdivided as follows: from 20 to 30 years — 10 persons, from 31 to 40 years — 10, from 41 to 50 years — 8 and over 50 years — 11 persons.

The subject's audiogram was taken, after which he was given the glucose powder (0.1 g), and then 30 minutes later a sound of frequency 1024/sec and intensity 50 db above the threshold of audibility was applied for 2 minutes to the right ear. The time of reversal of adaptation to a tone of 1024/sec was then measured in the same ear. After resting for one hour and reapplication of the excessive sound, the other ear was tested. After 4-5 days observations were carried out by the same method but after the preliminary administration of a mixture of glucose (0.1 g) and dibasol (0.01 g). In this series of observations too, the subjects were unaware of the composition of the powders.

The results of the investigation of the time of reversal of adaptation in the second series are shown in the figure. Here are shown the mean values of the time of reversal of adaptation for the 4 groups of subjects: those with normal hearing, those with diseases of the auditory nerves, those with stage III otosclerosis and those with stages I and II otosclerosis.

It will be seen that the greatest value of the time of reversal of adaptation (48 seconds) was found in the group of subjects with diseases of the auditory nerves. In the group suffering from stage III otosclerosis the mean value of the time of reversal of adaptation (45 seconds) was longer than that in stages I and II of otosclerosis (38 seconds). The time of reversal of adaptation was least in healthy subjects (18 seconds).

After administration of a single dose of 10 mg of dibasol, the average value of the time of reversal of adaptation (45 seconds) was decreased in all 4 groups of subjects. Its maximum decrease as a result of the drug was found in patients with stages I and II of otosclerosis (by 14 seconds), and its minimum in stage III otosclerosis (by 7 seconds). The degree of shortening of the time of reversal of adaptation in the group with diseases of the auditory nerves was insignificant (9 seconds). The average shortening of the time of reversal of adaptation in the group of healthy subjects was 11 seconds.

Our results confirm those of G. M. Komarovich's research [4], that the time of reversal of adaptation is longer in lesions of the nervous apparatus of the auditory analyzer, and also O. F. Zakharova's findings that during otosclerosis there is observed not only a disturbance of the conduction of sound but also a well-marked state of inhibition of the cortical division of the auditory analyzer, and this is diminished by dibasol. Accordingly, we consider it desirable to use dibasol in order to ascertain the character of the state of inhibition of the central divisions of the auditory analyzer, and hence to decide whether or not to operate in a certain stage of otosclerosis.

Our results concerning the property of dibasol, in doses of 5 and 10 mg, to shorten the time of reversal of adaptation after an excessive noise suggest that the drug may prevent the development of inhibition in the auditory analyzer and bring about a more rapid removal of the inhibition. This drug may therefore be recommended in persons exposed to the action of prolonged and intensive noise. Dibasol may also be used therapeutically in certain forms of deafness — neuritis, otosclerosis and so on; in these cases functional disturbances of the nature of obstructive inhibition are often observed along with organic disturbances in the auditory analyzer.

SUMMARY

The author studied the effect of dibasol on the adaptive changes of hearing in healthy individuals and in patients suffering from diseases of the auditory nerves and otosclerosis. As established, dibasol reduced the level of the maximal loss of the auditory sensitivity and the period of reverse adaptation. On these grounds the author recommends dibasol administration to persons exposed to noise for prolonged periods of time and suffering from some forms of a partial loss of hearing.

LITERATURE CITED

[1] N. N. Anosov and M. A. Rozin, Proserine, Eserine and Dibasol and Their Use in Neuropathology, Leningrad, 1956 [In Russian].

[2] G. V. Gershuni, Adaptation of the auditory function during the action of sound . Problems of Physiological Acoustics, Part 1, pp. 5-20. Moscow — Leningrad, 1949 [In Russian].

[3] G. I. Grinberg and F. Kh. Fridlyand, Analysis of adaptive changes in the sensation of hearing in the light of I. P. Pavlov's teaching . Transactions of the Naval Medical Academy, Vol. 39, pp. 355-361. Leningrad, 1952 [In Russian].

[4] G. M. Komarovich, Adaptation to intense sounds in diseases of the middle and inner ear . Problems of Clinical Physiology in Otolaryngology, pp. 19-29. Leningrad, 1955 [In Russian].

[5] N. V. Lazarev and M. A. Rozin, The action of drugs on the injured nervous system . Fiziol. Zhurn. SSSR, 40, 142-147 (1954).

[6] O. L. Nemtsova, Byull. Éksptl. Biol. i Med., 1, No. 6, 442-444 (1936).

[7] M. A. Rozin, Dibasol and inhibition . Proceedings of a Conference on the Experimental and Clinical Study of Dibasol, pp. 9-19. Leningrad, 1956 [In Russian].

THE EFFECT OF SALTS OF COPPER AND MANGANESE ON THE CHOLINERGIC SYSTEMS OF THE HEART

V. S. Raitses and Ya. V. Ganitkevich

From the Department of Normal Physiology (Head — Docent V. S. Raitses) of the Stanislav Medical Institute (Director — Docent G. A. Babenko)

(Received September 9, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

Copper and manganese are known to possess parasympatheticotropic activity. Copper chloride, for instance, depresses the work of the heart, and moreover, the degree of this action depends on the concentration of copper; copper salts in low concentrations increase, and in high concentrations decrease the inhibitory action of the vagus nerve on the frog's and rabbit's heart [7]. Potassium permanganate is another substance with a depressing action on the work of the isolated heart [6]. Many authors have shown that manganese and copper salts intensify the hypoglycemic action of insulin, diminish adrenalin and alimentary hyperglycemia, lower the blood sugar level in normal and diabetic subjects, and so on [1, 2, 8 and others]. The authors cited do not, however, explain the mechanism of the biological action of copper and manganese salts.

In the present work we attempted to discover the effect of salts of copper and manganese on the regulation of the cardiac activity, by studying the level of excitation of the cholinergic systems of the heart.

EXPERIMENTAL METHOD

The experiments were carried out on the frog's heart, isolated by Straub's method, during the autumn-winter period. Solutions of copper and manganese chlorides were made up in Ringer's solution before the beginning of the experiment. In this research we used copper salts in concentrations (calculated as the pure metal) of 1×10^{-8} to 1×10^{-5} and manganese salts in concentrations of 5×10^{-8} to 1×10^{-4}.

EXPERIMENTAL RESULTS

In the first series of experiments we studied the effect of these salts of copper and manganese on the frequency and amplitude of the cardiac contractions. It was found that the copper solutions in dilutions of 1×10^{-8}, when acting for a considerable time, caused no appreciable changes in the mechanogram of the heart. With a concentration of copper of 1×10^{-7} a gradual and very slight decrease was observed in the amplitude of the cardiac contractions, which reached only 20-30% of the initial value after 5 minutes. The amplitude of the cardiac contractions was sharply decreased as a result of administration of copper in a concentration of 1×10^{-6}. Under these conditions both a weakening of the systole and a decrease in the diastolic relaxation were observed, although the latter was rather less apparent. The height of the cardiac contractions fell by 50% in the course of a minute. The heart rate remained as before even when considerably weakened. Rinsing the heart with Ringer's solution restored the activity of the heart to its initial level with varying speed.

Investigation of the changes in cardiac activity under the influence of manganese salts showed that the prolonged action of manganese in a dilution of 5×10^{-8} caused a very slight fall in the amplitude of the cardiac contractions of not more than 20% in the course of 5 minutes. Under the influence of a 5×10^{-7} solution of

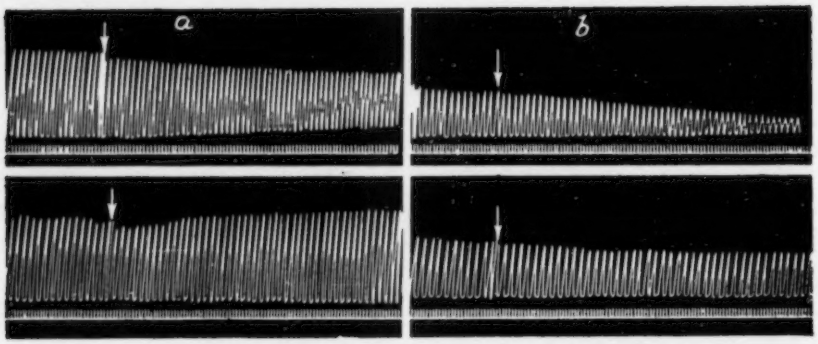


Fig. 1. The action of copper and manganese salts on the isolated frog's heart. a) action of copper (1×10^{-7}) after perfusion with Ringer's solution (I) and after atropinization of the heart (II); b) action of manganese (5×10^{-7}) after perfusion with Ringer's solution (I) and after atropinization (II) (the arrows indicate the beginning of perfusion with the test solution).

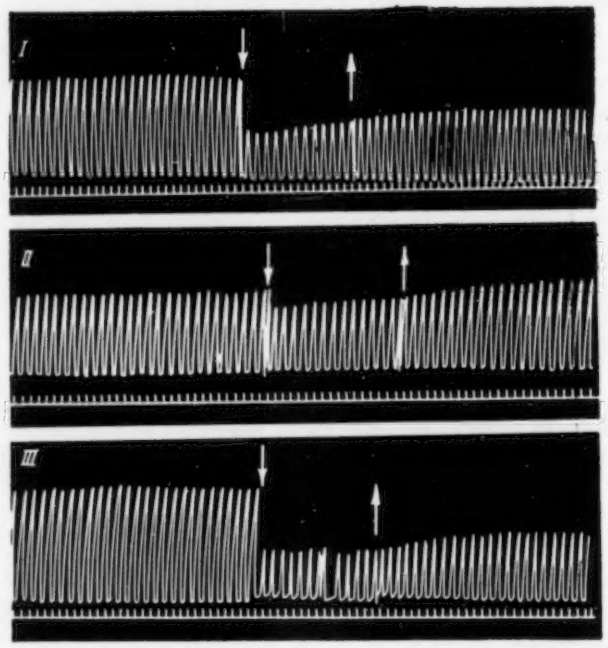


Fig. 2. Changes in the inhibitory action of acetylcholine on the heart under the influence of copper and manganese salts. I) action of 1×10^{-8} acetylcholine on the heart in control experiments; II) action of the same concentration of acetylcholine after perfusion of the heart with 1×10^{-7} copper solution for 5 minutes; III) the same after perfusion of the heart with 5×10^{-8} manganese solution for 5 minutes.

manganese the weakening of the cardiac contractions also developed gradually, but it was more pronounced and reached on the average 50% (Fig. 1, b, I). In a concentration of 1×10^{-6}, manganese caused a rapid fall in the amplitude of the cardiac contractions of 60-70% of their initial value, on account of a diminution of both systole and diastole. The heart rate was appreciably unchanged in all the experiments.

The electrocardiogram of the isolated heart under the influence of copper and manganese salts in the above concentrations showed no specific changes in its automatism and conductivity.

In the second series of experiments we studied the effect of copper and manganese salts on the heart aganist a background of the action of atropine. A 1: 10 000 solution of atropine was applied for 5 minutes, after which the effect of copper or manganese was tested. It was found that copper, in a dilution of 1×10^{-7}, did not cause any decrease in the amplitude of the contractions of the atropinized heart (Fig. 1, a II). In a concentration of 1×10^{-6}, copper led to an insignificant depression of the action of the heart in a minority of the experiments and only in a dilution of 1×10^{-5} did it constantly evoke a decrease in the amplitude of the contractions by 25-30%. In order to obtain an effect on the atropinized heart it was thus necessary to increase the concentration of copper almost a hundredfold.

Similar results were obtained by the action of manganese on the atropinized heart. During the action of manganese in a concentration of 5×10^{-8} to 1×10^{-5} the activity of the atropinized heart was completely unaltered (Fig. 1, b, II). Only in a dilution of 1×10^{-4} did manganese cause a gradual decrease of 50% in the amplitude of the cardiac contractions. It is clear from these results that the concentrations of manganese which depress the activity of the atropinized heart were increased roughly 200 times. From 10 to 30 minutes after rinsing the atropine from the heart, the previous sensitivity of the heart to copper and manganese was gradually restored.

In the third series of experiments we investigated the changes in the reaction of the heart to acetylcholine under the influence of copper and manganese salts. The solution of acetylcholine 1×10^{-8}, which we injected into the cannula, acted on the heart for 20 seconds. In most experiments in which copper solutions acted on the heart some degree of weakening of the reaction of the heart to acetylcholine was observed, depending on the concentration and the duration of action of the copper. Whereas in the control experiments the introduction of a 1×10^{-8} solution of acetylcholine caused, in 2-5 seconds, a decrease of 50-70% of the initial value in the amplitude of the cardiac contractions, after the prolonged action of high dilutions of copper salts (1×10^{-7}) or after the action of higher concentrations for a short period of time (up to 5 seconds), the same solution of acetylcholine caused a decrease of 20-40% in the amplitude of the cardiac contractions. The more prolonged action (10-15 minutes) of high concentrations of copper (1×10^{-5}), accompanied by marked changes in the mechanogram of the heart, completely abolished the reaction of the heart to acetylcholine (Fig. 2). In two experiments a small increase was observed in the cardiac contractions in response to the injection of acetylcholine. During the action of small concentrations of copper (1×10^{-7}), in some experiments a considerable increase was observed in the effect from the injection of acetylcholine.

Rinsing the heart with Ringer's solution after the action of small concentrations of copper caused the partial restoration of its reaction to acetylcholine, whereas after giving high concentrations of copper the absence of a reaction to acetylcholine was irreversible.

The foregoing refers to the effect of acetylcholine in dilutions of 1×10^{-8} to 1×10^{-9}. Increasing the concentration of acetylcholine a hundredfold causes arrest of the heart irrespective of the preliminary action of copper.

In order to test the character of the interaction between copper salts and acetylcholine, we carried out control experiments. After testing the reaction of the heart to acetylcholine and washing this out, we replaced the Ringer's solution in the cannula with a mixture of copper and acetylcholine solutions prepared 5-10 minutes before administration (in the same concentrations as in the previous test). It was found this mixture caused the same depression of the activity of the heart as did acetylcholine alone at the beginning of the experiment.

The study of the effect of manganese salts on the sensitivity of the heart to acetylcholine showed changes of an opposite character. Manganese in a dilution of 5×10^{-8}, which by itself did not appreciably alter the amplitude of the cardiac contractions, caused an obvious increase in the inhibitory action of acetylcholine. The decrease in the amplitude of the cardiac contractions in response to injection of acetylcholine after the action of manganese developed more quickly and was from 2 to 4 times more pronounced than that in response to injection of acetylcholine alone. The activity of the heart was also restored more slowly after the acetylcholine had been rinsed out (Fig. 2, III). With an increase in the concentration of manganese, its strengthening action on the inhibitory effect of acetylcholine was enhanced.

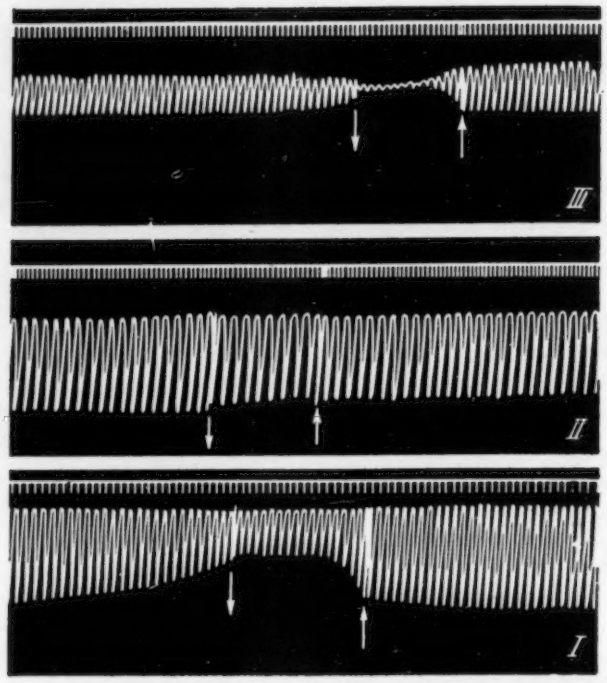


Fig. 3. The effect of cysteine on the cholinolytic action of copper. 1) action of 1×10^{-8} acetylcholine on the heart; II) action of the same concentration of acetylcholine after preliminary perfusion of the heart with a 1×10^{-7} copper solution; III) the same after preliminary perfusion of the heart with a 1×10^{-7} copper solution and 1% cysteine.

In the fourth series of experiments we studied the effect of compounds which acted as donors of sulfhydryl groups on the action of copper salts. As compounds of this type we used cysteine and urea which, from findings in Kh. S. Koshtoyants's [4] laboratory, restore the sensitivity of the heart to neurohumoral influences when it has been altered by blocking of the sulfhydryl groups by the thiol poisons. For this purpose we first acted on the heart with copper solutions, leading to a decrease in the reaction of the heart to acetylcholine or to its disappearance. Against this background, the copper solution in the cannula was replaced by 1% solution of cysteine, and the action of acetylcholine was again tested after 30-60 seconds. It was found that, after the action of cysteine solution, acetylcholine caused inhibition of the action of the heart, just as in the control experiments (Fig. 3). At the same time it has to be pointed out that after the action of urea (1-5% solutions) we were unable to observe any recovery of the reactivity of the heart to acetylcholine after its depression of copper salts.

Our investigations showed that copper and manganese salts, in low concentrations, not only affect the activity of the heart but also cause marked changes in the reactivity of the heart to acetylcholine. Both elements show a similar negative, inotropic action on the heart, characterized both by depression of systole and by incomplete relaxation during diastole. The fact that this effect was abolished by preliminary atropinization of the heart indicates its vagotropic character, i.e., this is in agreement with the reports in the literature of parasympathicotropic action of copper and manganese. Investigation of the changes in the reactivity of the heart to acetylcholine, however, revealed a difference in the mechanism of action of copper and manganese. It is clear from the results described that manganese, while it exerts a vagotropic effect on the heart, at the same time increases the level of excitation of its cholinergic systems. Copper, however, which also exerts a vagotropic effect, leads to a sharp depression of the level of excitation of the cholinergic systems. The experiments with cysteine showed that the depression of the level of excitation of the cholinergic system is connected with the blocking of the sulfhydryl groups by copper. Our results are in agreement with experimental findings that copper accelerates the oxidation of sulfhydryl into disulfide groups [3].

SUMMARY

Copper and manganese salts, in small concentrations (5×10^{-8} to 1×10^{-6}), thus causes a decrease in the amplitude of the contractions of the isolated frog's heart; under these circumstances the systolic contractions is weakened and the diastolic relaxation is diminished. The heart rate is unaffected. This action of copper and manganese salts does not take place after preliminary atropinization of the heart. The action of copper is to cause a decrease in the reactivity of the heart to acetylcholine, connected with blocking of the sulfhydryl groups by the copper; manganese, on the contrary, increases the sensitivity of the heart to acetylcholine.

LITERATURE CITED

[1] F. Ya. Berenshtein, Uspekhi Sovremennoi Biol., 2, 178 (1950).

[2] F. Ya. Berenshtein, Uspekhi Sovremennoi Biol., 25, 2, 203 (1948).

[3] A. O. Voinar, The Biological Role of Trace Elements in Animals and Man. Moscow, 1953 [In Russian].

[4] Kh. S. Koshtoyants, The Proteins, Their Metabolism and Its Nervous Control. Moscow, 1951 [In Russian].

[5] S. G. Sidorova and O. D. Tsvetkov, Farmakol. i Toksikol., 3, 5, 27 (1940).

[6] T. I. Tsobkallo, Fiziol. Zhurn. SSSR, 18, 6, 920 (1935).

THE DELIBERATE CULTIVATION OF ANTITUMOR PROPERTIES IN BACTERIA*

I. I. Oleinik and V. N. Yagodinskii (Leningrad)

(Received June 30, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

Data published in the literature in recent years have shown beyond doubt the fact that certain microorganisms exist in nature, the products of whose vital activity have a selective action on the malignant tumors of animals and man [2, 3].

The search for such microorganisms is made much more difficult, however, by the lack of any simple and speedy method of selection of producers of antitumor substances [4].

In the present work we have attempted, by taking advantage of the powers of adaptation of microorganisms, to seek another means of developing antitumor properties in bacteria whose initial, naturally occurring strains do not possess such properties [1, 5, 6].

EXPERIMENTAL METHOD

We used strains of proteolytic bacteria – Proteus vulgaris (isolated from soil) and Bacillus pyocyaneus (isolated from pus). A pure culture was transferred by means of a sterile loop from a solid nutrient medium (Endo's) to a flask containing 200 ml of sterile physiological saline. As a source of nitrogen for the proteolytic bacteria, to the flask was added 0.7-1.0 g of freshly minced tissue from a Crocker's mouse sarcoma, washed twice with sterile physiological saline. The flask was incubated at 37°. After 48 hours the culture was used to seed another flask, perpared the previous day by the same method. From each culture 90 such subcultures were taken. By means of a Seitz filter (disk SF) filtrates of 7-day cultures were obtained and these were used in the experiment.

The experimental animals were white mice. Using a needle with a stilet, small fragments of a Crocker's sarcoma were implanted beneath the skin of the dorsal region of the animals. Each strain of microorganisms was tested on 110 animals.

Sterile filtrate of one of the cultures, in a volume of 0.5 ml, was injected into the animals of the first experimental group on the day of transplantation of the tumor, and into the animals of the second group 5 days after transplantation of the tumor. Filtrate was injected three times into the animals of the third group: on the 1st day after transplantation (1 ml), on the 5th day (0.5 ml) and on the 10th day (0.3 ml). The animals of three control groups were injected with sterile filtrate of a 7-day infusion of tumor tissue in sterile physiological saline; the animals of the first group – on the day of transplantation of the tumor (0.5 ml), of the second group – 5 days after transplantation of the tumor (0.5 ml) and those of the third group – three times: on the 1st day (1 ml), on the 5th day (0.5 ml) and on the 10th day (0.3 ml). The fourth control group of animals were injected with a sterile 7-day filtrate of the original strain of one of the cultures (on the 1st, 5th and 10th days, in doses of 1.0, 0.5 and 0.3 ml respectively); finally, a fifth group was injected with sterile physiological saline. The times and doses were the same as for the fourth group.

* The authors express their deep gratitude to Prof. A. A. Sinitskii, Corresponding Member AMN SSSR M. M. Maevskii and Honored Scientist Prof. P. N. Kashkin, who acted as scientific consultants in the performance of this research.

TABLE 1

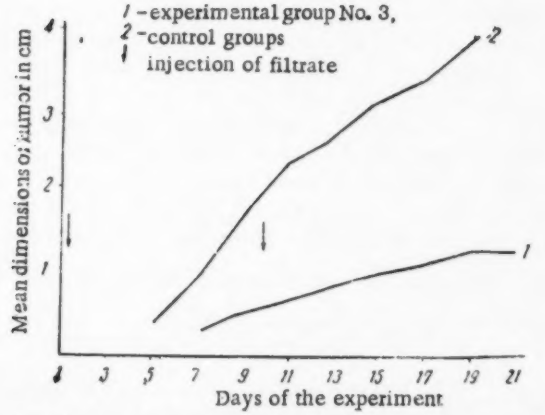
The Effect of Filtrate of a Test Strain of Proteus vulgaris on the Development of a Crocker's Sarcoma in Mice

Group of mice		Number of mice in group		Tumors completely absorbed	
experiment	control	experiment	control	experiment	control
First	First	20	10	5	—
Second	Second	20	10	2	—
Third	Third, fourth, and fifth	20	30	11	—
	Total	60	50	18	

TABLE 2

The Effect of Filtrate of a Test Strain of Bacillus pyocyaneus on the Development of a Crocker's Sarcoma in Mice

Group of mice		Number of mice in group		Tumors completely absorbed	
experiment	control	experiment	control	experiment	control
First	First	20	10	6	—
Second	Second	20	10	3	—
Third	Third, fourth and fifth	20	30	15	
	Total	60	50	24	—



The effect of filtrate of the test strain of Proteus vulgaris on the development and growth of a Crocker's sarcoma in mice. 1) experimental group No. 3, 2) control groups, ↓– injection of filtrate.

TABLE 3

The Effect of Filtrate of a Deliberately Cultivated Strain of B. pyocyaneus on the Success of Transplantation of a Crocker's Sarcoma in Mice

Material used for preparation of the tumor suspension	Number of mice		
	altogether in experiment	with tumor	without tumor
Sterile filtrate of deliberately cultivated strain of B. pyocyaneus	10	—	10
Sterile filtrate of original strain of B. pyocyaneus (control)	10	10	—
Sterile physiological saline (control)	10	10	—

TABLE 4

Loss of Antitumor Activity of a Filtrate of the Test Strain of B. pyocyaneus after Contact for 8 Hours with Cells of a Crocker's Sarcoma in Mice

Material used for preparation of the tumor suspension	Number of mice		
	altogether in experiment	with tumor	without tumor
Sterile filtrate of test strain of B. pyocyaneus after contact with tumor for 8 hours	10	9	1
Sterile filtrate of test strain of 7-day culture of B. pyocyaneus (control)	10	0	10
Sterile filtrate of test strain of B. pyocyaneus after contact with cardiac muscle for 8 hours (control)	10	7	3
Sterile physiological saline (control)	10	10	-

EXPERIMENTAL RESULTS

In the experiments using filtrates of the test strain of Proteus vulgaris, 36 mice of the experimental groups survived for 50 days from the beginning of the experiment, and in 18 of these animals the tumors were completely absorbed (Table 1). In none of the control groups was absorption of the tumors observed. The best results were obtained in the third group, after three injections of filtrate. Of 20 mice in this group, 16 were still alive after 50 days, and only 5 of these had tumors of small dimensions. Differences were also noted in the times of appearance and in the dimensions of the tumors in the animals of the experimental and control groups. In the mice of the control groups the tumors as a rule, could be palpated as small granules at the site of implantation on the 5th-7th day after inoculation of the sarcoma, and they reached their largest size on the 20th-25th day. In the experimental groups receiving three injections of filtrate, the developing tumors reached an appreciable size only at the end of the 2nd week, i.e., the time of manifestation of the tumor was delayed approximately 2-3 times. These tumors were also much smaller than those observed in the animals of the control groups (see figure).

Experiments using filtrate of the test animals of Bacillus pyocyaneus gave analogous results (Table 2).

In order to obtain data to confirm the selectivity of action of the culture filtrates on tumor cells in mice in vivo, 2 experiments were carried out.

The first experiment was conducted on 10 white mice into which a Crocker's sarcoma was implanted subcutaneously in the dorsal region in the form of a tumor suspension in sterile filtrate of a 7-day culture of the test strain of B. pyocyaneus. As controls we used mice into which was implanted a tumor suspension in sterile physiological saline and in sterile filtrate of the original strain of the 7-day culture. The results of the experiments were read on the 10th day.

It is apparent from Table 3 that no tumor development was observed in the experimental group of mice, whereas in the controls, the original strain did not produce a fall in the percentage of successful transplantation of the tumor. This indicates the presence of a direct action of the filtrate of test strain of B. pyocyaneus on the sarcoma cells, depriving them of their power of transplantation.

In the second experiment, minced Crocker sarcoma tissue was placed in sterile filtrate of the test strain of B. pyocyaneus, and left for 8 hours at a temperature of +4°, with uniform mixing. The filtrate was then freed from tumor particles by contrifugation, and in it was prepared a suspension of fresh tumor tissue of a sarcoma destined for transplantation into the animals of the experimental group. The control animals were injected with a tumor suspension prepared as indicated in Table 4.

On the 15th day of observation it was found that the filtrate of the test culture produced practically no fall in the percentage of successful transplantation after preliminary contact with tumor tissue. Evidently during the time of contact, a selective adsorption by the tumor cells of the active antitumor principle contained in the culture filtrate took place.

SUMMARY

Experiments were performed with the cultures of Proteus vulgaris and Bacillus pyocyaneus, the initial strains of which did not posses natural antitumor properties.

These bacteria were cultured for a long period in a medium in which Crocker's sarcoma cells formed the source of nutritive substances. This resulted in the appearance of certain substances among the metabolites of the bacteria able to selectively depress the development and the growth of Crocker's sarcoma in mice.

LITERATURE CITED

[1] O. E. Vyazov, Immunology of Normal and Malignant Tissues, p. 254. Moscow, 1956 [In Russian].

[2] G. F. Gauze, Antibiotics, p. 103. Medgiz, 1956 [In Russian].

[3] N. G. Klyueva and G. I. Roskin, Antibiotics against Cancer, p. 5. Moscow, 1956 [In Russian].

[4] M. M. Maevskii, Antibiotics, pp. 110-117. Medgiz, 1956 [In Russian].

[5] I. G. Shiller, Deliberate Antagonism of Microorganisms, p. 113. Kiev, 1952 [In Russian].

[6] A. E. Moore, Cancer Res., 1951, N. 11, p. 269.

THE IMMUNO-ADAPTIVE STAGE IN RATS IN RELATION TO NORMAL AND HETEROLOGOUS CELLS

J. Grozdanovic and A. Puza*

From the Division of Experimental Biology and Genetics of the Biological Institute of the Academy of Sciences of Czechoslovakia (Prague) and the Department of Biology of the Faculty of Medicine of the Komensky University (Kosice)

(Received December 27, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

According to data in the literature [1, 2, 3, 5], it is possible to induce in rats an immunological adaptation in relation to heterologous antigens (of mouse, rabbits or man) both during embryogenesis and in the early postembryonic period. We do not yet know for how long in the early postembryonic period the newborn rats preserve their powers of immunological adaptation. There are reports [4] that in the duck the stage of adaptation towards heterologous antigens lasts from the 6th to the 13th day after hatching.

In the present investigation we studied the immuno-adaptive stage in rats in relation to normal heterologous cells.

EXPERIMENTAL METHOD

Newborn rats, aged under 24 hours and 2, 3, 4, 5, 10, 15 and 20 days, were given a single subcutaneous injection of 0.2 ml of a 40% suspension of thrice-washed rabbits' red cells in the dorsal region. After 2 months the rats were immunized intraperitoneally with three doses of rabbits' red cells in the form of a 40% suspension (the first two doses of 0.5 ml each at intervals of 24 hours, and the third dose of 0.5-0.8 ml on the 4th-7th day after the second dose).

The second group of rats, also aged under 24 hours, received subcutaneous injections of 0.1 ml of citrated rabbits' blood. As in the preceding group, the rats were immunized at the same age with three intraperitoneal injections (each of 1 ml of citrated rabbits' blood at intervals of 2 weeks between doses).

The serum used for the determination of the agglutinins and their titers was taken from all the animals on the 9th-10th day after injection of the last immunizing dose. Before immunization, the serum of the experimental and control animals was investigated for the persence of natural heteroagglutinins against rabbits' red cells.

The rats were reimmunized on attaining sexual maturity and a weight of over 150 g, the methods of immuization, taking the sera and determining the agglutinin titer being exactly the same as at the first immunization.

In precisely the same way a 40% suspension of washed red cells from white mice was injected. The mouse red cells were injected into rats in a dose of 0.1-0.2 ml within 24 hours of birth and then at the age of 36 hours, and 5, 9 and 15 days. On attaining a weight of 50 g these animals were immunized intraperitoneally with three doses, each of 1 ml of citrated blood, at intervals of 3 days.

The animals which were injected with the suspension when less than 24 hours old were reimmunized 4 weeks

* With the technical collaboration of H. Zelenkova, I. Molnar and I. Susova.

General Scheme of the Results of Immunological Adaptation of Rats in Relation to Heterologous Red Cells

Age of animal and mode of injection of antigen	Mouse red cells	Homogenized mouse tissues	Rabbits' red cells	Human red cells
Single injection:				
to embryos 3-4 days before birth	+	+	+	±
to newborn rats	+	+	+	–
24 hours after birth	+	+	+	–
36 hours after birth	–	–	–	
5 days after birth	–	–	–	
9 days after birth	Immunity	Immunity	Immunity	
15 days after birth	"	"	Immunity	
20 days after birth			"	
Second injection:				
to embryos 3 days before birth				
to newborn rats 1-5 days after birth				+
to newborn rats on the 1st-16th day of life				+

Legend: + production of statistically significant tolerance; ± production of tolerance not statistically significant; – no tolerance produced.

after the last dose of the first immunization by the method indicated. In these cases too, serum for the estimation of the immune agglutinins was taken on the 10th day after the last immunizing dose.

EXPERIMENTAL RESULTS

The effect of injection of mouse red cells in the postembryonic period was investigated in 19 newborn rats. A statistically significant decrease was found in the formation of immune agglutinins by comparison with 15 control rats both after primary immunization and reimmunization.

The decrease in the formation of immune heteroagglutinins against rabbits' red cells was observed in 22 experimental animals which received injections of antigen when under 24 hours of age. On comparison of the mean titers with those of a control group of 24 young rats of the same age, taken in the majority of cases from the same litters as the experimental animals, a statistically significant difference was found between the mean titers in respect to a decrease in their ability to form antibodies. Before reimmunization no convincing difference could be found between the mean titers in the experimental and control animals. The same thing was observed after reimmunization. Immunological adaptation to the rabbits' red cells which was created in the newborn rats was weaker than that induced in embryos.

In agreement with the results obtained in the preceding investigations, we were unable to demonstrate the presence of any natural heteroagglutinins in the rats. Injection of mouse red cells to young rats 36 hours after birth and on the 5th day of life did not induce adaptation, but after injections of mouse red cells on the 9th day of life, in some experimental immunized animals higher titers of immune heteroagglutinins were found than in the control animals.

We obtained values for experimental and control rats which were injected with rabbits' red cells on the 2nd-20th day of life. The agglutinin titers before and after immunization showed that injection of cellular antigen on the 2nd-5th day of life had no essential effect on the formation of agglutinins in adult rats. This is a sort of zero period. Beginning on the 10th day of extrauterine life, injection of antigens now caused the appearance of immune agglutinins in some rats before immunization, and the appearance of higher titers than in the control animals – after immunization.

Our experiments showed that the stage of immunological adaptivity in rats in relation to mouse and rabbits' red cells when giving in a constant dose (0.1-0.2 ml of a 40% suspension) ended on the first day after birth. By using a phylogenetically more distant antigen, for example human red cells, injection of the same volume of suspension to animals of the same age, however, did not now cause adaptation. In order to produce this degree of adaptation in these cases a far greater dose of antigen was required [3].

Between the 2nd and 5th day of life the volume of antigen used caused neither adaptation nor an immune reaction; starting on the 9th-10th day it caused antibody formation in certain animals.

The table gives in schematic form an assessment of our findings concerning the immunological adaptation of rats in relation to heterologous antigens.

The conclusion of the stage of immunological adaptivity must always be determined in respect to a given antigen and to a definite dose.

The characteristics and the duration of the so-called zero period, in which neither adaptation nor immunity is observed, remain uncertain. From the results of our previous experiments on the study of the immunological adaptation of rats to human red cells, it follows that by using a particular antigen in doses not reaching a certain threshold value, the "zero period" may be shown as that period of development of the animals in which a higher dose will still induce adaptation.

Thus the duration of the so-called zero period is also relative, i.e., it always depends on the particular antigen and the particular dose. This also follows from experiments studying homologous adaptation, which showed that it was possible to produce tolerance in rats until the 14th day of extrauterine life [6], i.e., when we obtained for the same dose of heterologous antigens either the zero period or a period of commencing antibody formation.

SUMMARY

The immuno-adaptive period in rats in relation to a single administration of mouse and rabbits' red cells ends during first day after birth. Administration of these red cells from the 2nd to the 5th day after birth causes neither immunological adaptation nor antibody formation. Beginning from the 9th or 10th day of life a single injection of the same doses causes antibody formation in some animals.

LITERATURE CITED

[1] W. Bolag, Experientia, 1955, 11, 227.

[2] J. Grozdanovic, Cs. biologie, 1956, 5, 300.

[3] J. Grozdanovic, Cs. biologie, 1957, 6. 87.

[4] V. Haskova, Cs. biologie, 1957, 6. 81.

[5] A. Puza and J. Molnar, Cs. biologie, 1956, 5. 304.

[6] M. F. Woodruff and L. O. Simpson, Brit. J. Exp. Path., 1955, v. 36. 494.

ONCOLOGY

CHANGES IN THE IMMUNOBIOLOGICAL PROPERTIES OF A TUMOR AFTER PASSAGE THROUGH A CHICK EMBRYO

T. A. Pokrovskaya

From the Laboratory of Noninfectious Immunology (Head – Prof. I. N. Maiskii) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received April 9, 1959. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

No unanimity exists in the literature on the question of the changes in the antigenic properties of transplanted tumors after passage through a chick embryo. The findings of a number of authors [1, 2, 5, 8] testify to changes in the biological and antigenic properties of tumors when cultivated in this medium.

Other workers [2, 4, 6, 7], on the other hand, deny that such changes may be produced.

In the present research we studied the immunobiological properties of the ascitic form of Ehrlich's adenocarcinoma after passage on the chorionallantoic membrane of the chick embryo.

EXPERIMENTAL METHOD

1. Passage of the tumor was carried out on the chorionallantoic membrane of 8-9 day-old embryos. The surface of the chorionallantoic membrane was exposed in sterile conditions, and at the place where there was greatest development of the vessels, 0.1 ml of ascitic fluid was applied, diluted 1:2. The opening in the shell was closed with a glass cover slip and sealed with paraffin wax. The embryos were examined and further transplantation carried out on the 14th-16th day of incubation. Growth of the tumor cells was observed in the course of seven passages, as confirmed histologically. After the seventh passage, growth of the tumor ceased.

2. The study of the antigenic structure of the transplanted tumor was made by means of the classical complement fixation reaction. As antigens we used a nontransplanted Ehrlich's adenocarcinoma and all the tumors obtained by passage (from the first to the seventh). Immune sera (against Ehrlich's adenocarcinoma, against the chorionallantois and against tumors after 5 passages) were always used in the same titer, namely 1:320.

3. The biological properties of the transplanted tumor was studied by transplantation back into mice. In the experiments we used male white mice weighing 18-20 g. The tumor was implanted subcutaneously in the dorsal region in a dose of 0.2 ml of a 10% suspension of tumor tissue. As controls we used mice which received injections of the same dose of a nontransplanted Ehrlich's adenocarcinoma.

The day of appearance of the tumors was noted and their dimensions on the 7th, 14th, 21th and 28th day after implantation were recorded. The size of the tumor was determined from the area of an ellipse. In all, 23 series of experiments were performed on 730 mice by this method.

EXPERIMENTAL RESULTS

In order to study the antigenic properties of the tumor after passage, two sera were used: a serum against Ehrlich's adenocarcinoma and a serum against the chorionallantois of a 15-day chick embryo. The reaction of the transplanted tumor with serum against Ehrlich's adenocarcinoma showed the extent to which the original antigenic composition was retained in the cells of the heterotransplanted tumor.

TABLE 1

Immunological Analysis of an Ehrlich's Adenocarcinoma after Passage through a Chick Embryo, by Means of the Complement Fixation Reaction

Dilutions of sera	Sera: against Ehrlich's adenocarcinoma — antigens: Ehrlich's tumor — nontransplanted	Ehrlich's tumor — passage I	Ehrlich's tumor — passage III	Ehrlich's tumor — passage V	Ehrlich's tumor — passage VII	chorion-allantois	Sera: against chorionallantois — antigens: chorion-allantois	Ehrlich's tumor — nontransplanted	Ehrlich's tumor — passage I	Ehrlich's tumor — passage III	Ehrlich's tumor — passage V	Ehrlich's tumor — passage VII	Sera: against tumor after 5th passage — Ehrlich's tumor — passage V	Ehrlich's tumor — passage III	Ehrlich's tumor — passage I	Ehrlich's tumor — nontransplanted	chorion-allantois
1:10	++++	+++	++	+	+	h	++++	h	h	+	+	+	++++	++	±	+	+
1:20	++++	+++	++	±	±	h	++++	h	h	±	+	+	++++	++	+	+	+
1:40	++++	++	+	h	h	h	++++	h	h	h	±	±	++++	+	h	+	+
1:80	+++	++	+	h	h	h	+++	h	h	h	h	h	++++	±	h	h	h
1:160	+++	+	h	h	h	h	+++	h	h	h	h	h	+++	h	h	h	h
1:320	++	+	h	h	h	h	++	h	h	h	h	h	++	h	h	h	h
1:640	+	h	h	h	h	h	±	h	h	h	h	h	+	h	h	h	h
Control		CHS	−	++++				CC-h		CS	−	h		CA-h			

Legend: ++++ complete inhibition of hemolysis; h, complete hemolysis; CHS, control of hemolytic system; CC, control of complement; CS, control of serum; CA, control of antigen.

TABLE 2

Incidence of Successful Retransplantation of the Tumor in Mice

passage	Experiment					Control				
	number of animals	mean area of tumor (in cm^2)				number of animals	mean area of tumor (in cm^2)			
		7th day	14th day	21st day	28th day		7th day	14th day	21st day	28th day
1st	50	0,06	1,06	2,64	3,55	50	0,06	1 06	2,57	3,6
2nd	50	0,023	0,4	1,19	2,4	50	0,014	0,73	1,75	3,04
3rd	50	0,018	0,18	0,6	1,51	50	0,069	0,64	2,05	3,51
4th	50	0,026	0,13	0,43	0,97	50	0,04	0,55	1,74	2,64
5th	30	No growth of tumor				30	0,07	1,01	2,15	3,01

The reaction with the serum against the chorionallantois enabled it to be judged whether the changes in the antigenic properties of the tumor was dependent on the presence of the tissues of the new host therein.

When studying the activity of the antigens obtained we took into consideration the limiting dilutions of the sera in which fixation to the extent of one plus sign could be observed. This was made easier by the fact that the sera which we used were all of the same titer, 1:820.

The immunological analysis on the transplanted tumor is shown in Table 1. It will be seen from Table 1 that after the 1st passage, the tumor gave a reaction of + in a dilution of 1:160 with serum against Ehrlich's adenocarcinoma. In the course of passage, however, the antigenic activity of the tumor fell slightly: after the 7th passage, the tumor reacted + with this serum only in a dilution of 1:10. In the reaction with serum against the chorionallantois, tumours after the 5th and 7th passages reacted + only in a dilution of 1:20. The nontransplanted tumor and tumors after the 1st to 4th passages did not react with this serum.

In the course of repeated passages on the chorionallantoic membrane, the Ehrlich's adenocarcinoma thus gradually lost the power to react with serum against this tumor.

At the same time, the absence of reaction with serum against the chorinallantois confirmed that the change in the original antigenic activity of the tumor in the complement fixation reaction was not connected with the presence of chorionallantoic tissue in the tumor.

Changes in the antigenic properties during passage were also confirmed by the complement fixation reaction with serum against the tumor after the 5th passage. (Because of the absence of large amounts of the transplanted tumor, it was possible to obtain serum against the tumor after only the 5th passage).

The results of this reaction are also shown in Table 1.

Serum against the tumor after the 5th passage reacted ++ in a dilution of 1:320 with homologous antigen.

Tumors after the 1st and 3rd passage reacted rather differently with this serum: the 3rd passage tumor reacted + in a dilution of 1:40, and the 1st passage tumor only in a dilution of 1:20.

As pointed out above, the study of the biological properties of the transplanted tumor was carried out by transplantation of the tumor back into mice. Table 2 gives in summarized form the results of all these experiments.

As is apparent from Table 2, in the course of repeated passage, the tumor gradually lost its ability to be transplanted into mice. Whereas in the first four passages the rats of successful transplantation of the tumor was still 100%, in the 6th passage it was zero.

The rate of successful transplantation of the tumor after the 5th passage into mice will be discussed below.

In the process of passage the tempo of growth of the tumor is modified as well as the rate of its successful transplantation, as may be seen from a comparative analysis of the dimensions of the tumor size at different

periods. The dimensions of the 1st passage tumor were in no way different from those of the control tumors. Starting with the 2nd passage, however, the tempo of growth of the tumor was slightly retarded.

The tumors appeared at the same time as those in the control animals, but then they grew more slowly; on the 28th day, the tumor in the experimental group had attained dimensions of 2.4 cm^2, whereas the area of the control tumors was 3.4 cm^2. Similar results were obtained from a study of the dimensions of the tumors after the 3rd and 4th passages.

Statistical treatment of the results showed that the difference between the dimensions of the tumors after the 3rd and 4th passages and those of the control tumors was significant, $P < 0.01$.

A somewhat different picture was obtained from the study of the rate of successful transplantation of the tumor after the 5th passage.

In the experimental group, the tumors appeared 7-8 days later than in the control group; on the 19th-26th day they attained an average diameter of 0.2-0.5 cm and then regressed for 1 to $2^1/_2$ months. The tumor after the 5th passage was transplanted into 135 animals, in 83% of which it was absorbed and in 8.8% it grew progressively and led to death of the animals. In 8.2% of mice transplantation of the tumor after the 5th passage was inconclusive.

The tempo of growth of the tumor after the 5th passage was retarded still further. Whereas the area of the tumors after the 3rd and 4th passages reached 0.18 and 0.13 cm^2 on the 14th day, the area of the tumor after the 5th passage did not exceed 0.11 cm^2. Retardation of the tempo of growth of the tumor after the 5th passage was also indicated by their later appearance by comparison with the tumors in the control group.

In the course of passage of an Ehrlich's adenocarcinoma on the chorionallantois of the chick embryo, changes were observed in the initial antigenic activity of the tumor and the rate of its successful transplantation in mice was diminished.

The results obtained show that during passage of the tumor on the chorionallantois of the chick embryo, a change takes place in its antigenic properties. This change in the antigenic properties coincides with changes in its biological properties.

SUMMARY

The author studied the antigenic and the biological properties of Ehrlich's ascitic adenocarcinoma grown on the chorionallantois of chick embryo. In the process of cultivation there was noted a change of the initial antigenic activity in complement fixation reaction and decrease of its ability to grow on mice. It is of interest to note that the changes of the antigenic properties of the cultivated tumor coincided with the change of its biological properties.

LITERATURE CITED

[1] V. A. Anan'ev and V. R. Obukhova, Zhur. Mikrobiol., Épidemiol. i Immunobiol., No. 11, 48 (1955).

[2] T. P. Konstantinova and Z. I. Rovnova, Byull. Éksptl. Biol. i Med., 45, No. 4, 112 (1958).*

[3] A. S. Kriviskii and L. F. Larionov, Arkh. Patol., 12, 3, 52 (1950).

[4] B. S. Ruchkovskii, Med. Zhur., 24, No. 6, 34 (1954).

[5] A. K. Shubladze, Zhur. Mikrobiol., Épidemiol. i Immunobiol., No. 11, 36 (1955).

[6] C. P. Dagg, D. A. Karnofsky and H. W. Toolah et al., Proc. Soc. Exper Biol and Med., 1954, v. 87, N. 1, p. 223.

[7] L. Korngold and R. Lipari, Cancer Res., 1955, v. 15, N. 3, p. 159.

[8] E. A. Mirand and J. G. Hoffman., Proc. Soc. Exper. Biol and Med., 1955, v. 88, N. 4, p. 656.

* Original Russian pagination. See C. B. Translation.

THE EFFECT OF ANTITUMOR SERUM ON THE MITOTIC ACTIVITY OF NEOPLASTIC AND NORMAL TISSUES IN THE MOUSE

S. V. Sukhorukikh

From the Laboratory of Noninfectious Immunology (Head — Prof. I. N. Maiskii) of the Institute of Experimental Biology (Director — Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received November 20, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

Research by V. N. Dobrokhotov [1] and our own previous investigations [2-4] have shown that 24 hours after administration of an antitumor serum, the intensity of cells division in an Ehrlich's adenocarcinoma is lowered. The antitumor serum does not produce (24 hours after administration) at the same time any essential changes in the mitotic activity of the epithelium of the cornea [4] or of the intestinal crypts [1]. It has also been shown [1] that antitumor serum, when injected in early periods of development of a tumor (24 hours or 4 days after implantation of the tumor material), had after 4-6 days a depressing effect on the mitotic activity of intraperitoneal nodes of an Ehrlich's adenocarcinoma. The trend of the changes in the mitotic activity of the tumor cells and also the mitotic activity of normal meristems were not studied by this author in these experimental conditions.

The aim of the present work was to ascertain the effect of antitumor serum on the changes in mitotic activity in a subcutaneous Ehrlich's adenocarcinoma in the period of its intensive development, and also in normal meristems (the epithelium of the cornea and of the crypts of the small intestine) at different times after the administration of antitumor serum. Since our previous findings [2-4] showed that other specific sera, such as the serum of a normal, nonimmune rabbit, have no depressing effect on the mitotic activity of tumor cells, these sera were not investigated in this particular experiment.

EXPERIMENTAL METHOD

We investigated 3 sera, obtained by the intravenous immunization of rabbits of the chinchilla breed with a saline extract of ascites cells of an Ehrlich's adenocarcinoma. The results of immunological testing of the sera by the complement fixation reaction at 37° were as follows: all 3 sera reacted with tumor antigen in a dilution of 1:400 +++; with antigen from the spleen of a healthy mouse the first serum reacted in a dilution of 1:100 +++, the second, in a dilution of 1:100 ++, the third, in a dilution of 1:200 ++; with antigen from the liver of a healthy mouse the first serum reacted in a dilution of 1:100 ++++ and further dilutions caused hemolysis; the second serum in dilutions of 1:100 and above gave hemolysis; the third reacted in a dilution of 1:100 ++++ and higher dilutions gave hemolysis.

The experiments were performed on 84 male white mice which were divided into 2 groups, each of 42 animals, on the 7th day after implantation of an Ehrlich's adenocarcinoma under the skin of the dorsal region. On the 7th, 8th and 10th days after implantation of the tumor, the mice of the experimental group were given a subcutaneous injection of 0.5 ml of antitumor serum in the dorsal region at some distance from the tumor. No serum was injected into the control group of mice. The mice of the experimental and control groups were then further subdivided into groups according to the time after the last injection of serum that the animals were sacrficed:

Time of sacrifice	Experiment	Control
After 1 day	I group ... 10 mice	II group... 10 mice
" 2 days	III " ... 10 "	IV " ... 10 "
" 4 "	V " ... 10 "	VI " ... 10 "
" 5 "	VII " ... 12 "	VIII " ... 12 "

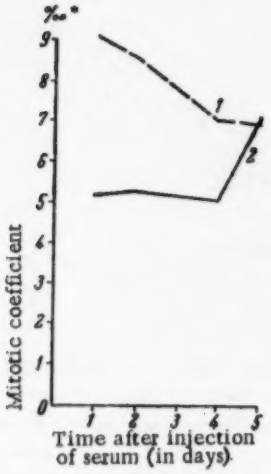


Fig. 1. Mitotic coefficient in an Ehrlich's adencarcinoma after injection of antitumor serum. 1) control; 2) experiment.

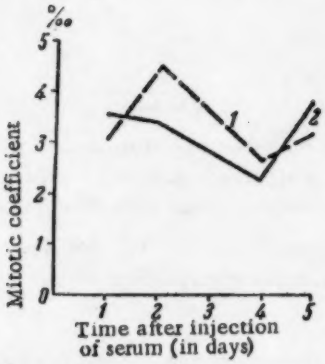


Fig. 2. Mitotic coefficient in the corneas of mice after injection of antitumor serum. 1) control; 2) experiment.

Animals of groups I and II thus were sacrificed on the 11th day of development of the tumor, those of groups III and IV on the 12th day, of groups V and VI on the 14th day and groups VII and VIII on the 15th day.

At each time of the investigation the mice of the experimental and control groups were sacrificed simultaneously, starting at 11 a.m.

The tumors and intestine were fixed in Carnoy's fluid, the cornea in a 5% solution of acetic acid in 70% alcohol. Sections of tumor and intestine were cut to a thickness of of 8μ, and total preparations of the cornea were made. In all cases the films were stained by Carazzi's hematoxylin.

The mitoses in the tumor were counted in 300 fields of vision, and in the cornea — on both sides of two perpendicular lines drawn in ink on the cover glass of the preparation. In each tumor an average of 60 000-70 000 cells was counted, and in the corneal epithelium — 30 000-35 000 cells. The number of mitoses in the intestine was determined in 50 crypts, cut longitudinally, in which roughly 3500 cells were usually counted. As a result of the counting of the mitoses in the phases of division, the coefficient K (ratio between the sum of the early phases of division to the late) and the mitotic coefficient MK (the ratio between the number of dividing cells and the total number of cells counted in the particular specimen, per 1000) could be calculated for each test object. The experimental results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

The counts of the mitotic activity in the tumors of all the experimental and control groups of mice showed that the mean percentage of the different phases of division was approximately equal for each group (P ≈ 3-6%, M ≈ 74-79%; A ≈ 2-4%; T ≈ 13-17%), as also was the coefficient K (from 4 to 5). The trend of the changes in the mean value of MK in the tumor in the course of the 5 days after the last injection of serum is indicated in Fig. 1.

*‰ = parts per thousand.

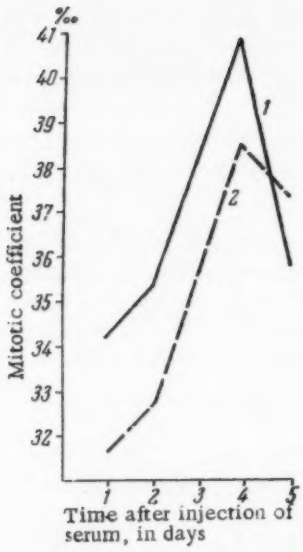


Fig. 3. Mitotic coefficient in the intestinal crypts of mice after injection of antitumor serum. 1) Experiment; 2) control.

It is clear that the value of MK in the subcutaneous Ehrlich's adenocarcinoma of the experimental mice was roughly the same on the 1st, 2nd and 4th day after injection of the serum (5.18; 5.26 and 5.05 per 1000) and only on the 5th day did it increase to 7.05 per 100 (for the interval between the 4th and 5th days p = 0.04).

The value of MK in the tumors of the control mice in the first 3 days of the investigation considerable exceeded the corresponding values inthe experimental group (9.07; 8.59 and 7.05 per 1000 respectively). At a later period of the investigation the value of MK in the tumors of the control animals was 6.95 per 1000, i.e., it was practically equal to the value of MK in the tumors of the experimental mice sacrificed on the same day (on the 5th day after injection of the serum). The difference between the values of MK in the experimental and control animals one day after injection of the serum was significant (p = 0.001), and after 2 days — P=0.022. On the 4th day after injection of the serum the difference between the values of MK in the experimental and control mice was less clearly shown, as the result of a slight fall in the value of MK in the tumors of the control animals (p = 0.041). Consequently, the mitotic activity of the tumor cells of a subcutaneous Ehrlich's adenocarcinoma was at a lower level in the course of the first 4 days (especially so during the first 2 days) after the injection of antitumor serum than in the control experiments.

The mean percentage of the different phases of division in the corneal epithelium of all the experimental and control groups of mice varied within narrow limits (P ≈ 17-25%; M ≈ 33-41%; A ≈ 5-9%; T ≈ 26-37%). The coefficient K in all the groups was also roughly identical (between 1.4 and 2.0). The changes in the mean values of MK in the corneal epithelium of the mice in the course of the 5 days after injection of the antitumor serum are illustrated in Fig. 2.

As is clear from Fig. 2, the value of MK in the corneal epithelium of the experimental groups of mice was roughly the same at all periods of the investigation (after 24 hours — 3.57 per 1000; after 2 days — 3.43 per 1000; after 5 days — 3.98 per 1000). The lowest value of MK (2.32 per 1000) was observed on the 4th day after injection of the serum (14th day of development of the tumor). The value of MK in the corneal epithelium of the control mice also varied between narrow limits (after 24 hours — 3.16 per 1000, after 2 days — 4.55 per 1000; after 4 days — 2.71 per 1000, after 5 days — 3.3 per 1000), and moreover the lowest value of MK (2.71 per 1000) was observed at the same period of the investigation as that in the experimental animals (14th day of development of the subcutaneous Ehrlich's adenocarcinoma).

The difference between the values of MK in the control epithelium of the experimental and control mice at each period of the investigation was not statistically significant. Consequently, the changes in the mitotic activity in the corneal epithelium of the experimental and control mice in the course of the 11-15 days of development of the adenocarcinoma were identical and did not depend on the effect of the antitumor serum.

The mean percentage of the various phases of division in the epithelium of the intestinal crypts of all the experimental and control groups of mice varied between narrow limits (P≈3-9%; M≈52-60%; A≈3-6%; T≈26-29%), as also did the coefficient K (betwen 1.3 and 2.3).

The curves showing the changes in the value of MK in the intestinal crypts on the 1st, 2nd, 4th and 5th days after injection of the serum are given in Fig. 3.

It will be seen that the value of MK was almost the same one and 2 days after injection of the serum (34.20 and 35.25 per 1000), rose to 40.8 per 1000 on the 4th day and then fell on the 5th day to 35.74 per 1000. However, the difference between the value of MK for these groups was not statistically significant. The changes in the value of MK in the intestinal crypts of the control mice were similar to those observed in the experimental

animals (31.67; 32.07; 38.48 and 37.31 per 1000), and moreover the highest value of MK (38.48 per 1000) was observed at the same period of the investigation as in the experimental mice. The differences between the values of MK of all the control groups were not statistically significant. Nor was the difference between the values of MK for the intestinal crypts of the experimental and control groups of mice at each period of the investigation statistically significant.

The small increase in MK in the intestinal crypts on the 4th day after injection of serum therefore did not depend on the injection itself, for a similar increase was observed in the control groups also at this period of the investigation.

The experimental results thus show that the depressing effect of antitumor serum on the mitotic activity of the tumor cells of a subcutaneous Ehrlich's adenocarcinoma lasts for 4 days after injection of the serum, and furthermore that a more intensive depression of cell division is observed during the first 2 days. The injection of antitumor serum in mice with a subcutaneously developing Ehrlich's adenocarcinoma causes no essential changes, during the 5 days after injection of the serum, in the mitotic activity in the epithelium of the cornea and the crypts of the small intestine of the experimental animals.

SUMMARY

This work deals with the study of the effect of antitumor serum on the dynamics of changes of mitotic activity in Ehrlich's subcutaneous adenocarcinoma, as well as in the epithelium of the cornea and crypts of the small intestine in the same experimental animals. The results of the experiment conducted on 84 male mice demonstrated that the antitumor serum has a depressing effect on the mitotic activity of Ehrlich's adenocarcinoma for a period of 4 days after the administration of the serum; the depression of cell division is more intense during the first 48 hours. There are no significant changes of mitotic activity in the corneal epithelium and crypts of the small intestine in mice with subcutaneously inoculated Ehrlich's adenocarcinoma for 5 days after the administration of the anti-tumor serum.

LITERATURE CITED

[1] V. N. Dobrokhotov, Byull. Éksptl. Biol. i Med., No. 3, 59-62 (1955).

[2] S. V. Sukhorukikh, Immunology of Normal and Malignant Tissues, pp. 85-93. Moscow, 1956 [In Russian].

[3] S. V. Sukhorukikh, Proceedings of a Scientific Conference on Technical Problems of the Immunology of Malignant Neoplasms, p. 26, Moscow, 1956 [In Russian].

[4] S. V. Sukhorukikh, Byull. Éksptl. Biol. i Med., 48, No. 7, 83-86 (1959).

THE EFFECT OF LATE REIMMUNIZATION ON THE PRODUCTION OF ANTITUMOR ANTIBODIES

P. P. Filatov

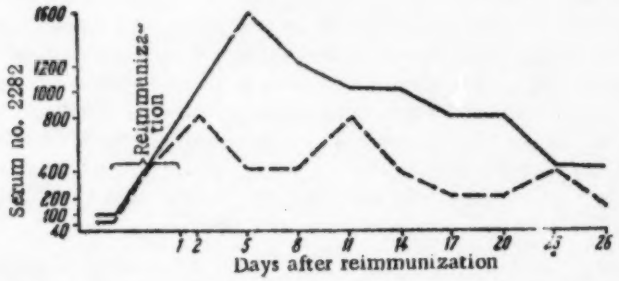
From the Laboratory of Noninfectious Immunology (Head – Prof. I. N. Maskii) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received April 6, 1959. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

The effect of repeated immunization on the immunological reactivity of the body after a long interval is a well-studied problem in infection immunology. The work of a number of authors [1, 2, 3] has shown that in response to late reinjections of antigens the antibody production (precipitins, agglutinins) is considerably increased by comparison with the height of the titer after primary immunization.

So far as the immunization of animals with tumor material is concerned, however, this problem has so far received insufficient attention.

The aim of the present work was to ascertain the effect of late reimmunization with antigens from tissue of Ehrlich's tumor of mice on the production of antibodies after a long interval.



The course of antibody production in rabbits after reimmunization with antigens from tissue of an Ehrlich's mouse tumor.
---- complement fixation reaction with tumor antigen.
—— complement fixation reaction with spleen antigen.

EXPERIMENTAL METHOD

Primary immunization was undertaken with saline extracts of the tissue of an Ehrlich's mouse adenocarcinoma (ascitic form) by the shortest scheme of 4 injections every other day.

Experiments were carried out on rabbits. The animals were divided into 5 groups, each of 4 rabbits. The first group of animals received injections of 0.2 mg (as protein) of antigen per course of immunization, the second

—2 mg, the third—12 mg, the fourth—40 mg and the fifth—100 mg per course. The antigens were injected intravenously. The quantity of protein was determined by Conway's nitrogen method. On the 5th day after the last injection of antigens, and thereafter every third or fourth day for 2 months, blood was taken from all the rabbits and sera prepared.

The results of the serological analysis of the sera obtained after primary immunization were published previously [4].

The rabbits were reimmunized with antigens from the same tumor $4^1/_2$ months after the conclusion of the primary immunization. The antugens were also injected intravenously—3 times on alternate days. The first group received 0.15 mg (as protein) per course of reimmunization, the second—1.5 mg, the third—9 mg, the fourth—30 mg and the fifth—75 mg. On the day before the beginning of injection of the antigens, on the day after the beginning of reimmunization and thereafter every third or fourth day for one month, blood was again taken and sera prepared for serological analysis.

The serological analysis of all the sera thus obtained was made by means of the classical complement fixation reaction at 37°.

EXPERIMENTAL RESULTS

Tests of the sera obtained by means of the complement fixation reaction showed that all the rabbits produced antitumor antibodies to roughly the same degree after reimmunization.

For instance, in the sera of animals reimmunized with antigens in a dose of 0.15 mg per course, the titer of antitumor antibodies varied from 1:1000 "++" to 1:1600 "++". In the sera of the animals of the second group reimmunized with antigens in a dose of 1.5 mg, the antibody titer varied within the same limits. In all the remaining groups of reimmunized animals, the titer of antitumor antibodies was roughly equal to the titer in the sera of the first 2 groups of rabbits.

In these experiments we were thus unable to detect any relationship between the immunological reactivity (the production of antitumor antibodies) and the doses of antigens injected, as was observed after the primary immunization.

Attention should be drawn to the fact that the titer of antitumor antibodies was twice as high after reimmunization as after primary immunization.

It is also interesting to observe that an increase in the titer of antitumor antibodies was found after reimmunization in all the animals. From an inspection of the course of production of anti-tumor antibodies, we noted that the growth of the antibody titer after reimmunization took place more rapidly than at the end of primary immunization. For instance, whereas after primary immunization the highest titer of antitumor antibodies was observed on the 9th-14th day, after reimmunization it reached its highest level on the 5th-8th day. For a clearer illustration of the course of antibody production, in the figure we show the most typical curve. The points on the curve indicate the complement fixation reaction with an intensity of "++". In order to determine the specificity of the sera obtained, the complement fixation test was set up not with antigens from tumor tissue, but also with antigens from liver and spleen tissue from normal mice. The curves depict the reaction with antigens from spleen tissue only, as being closest in an antigenic respect to tumor.

Besides the accelerated antibody production already mentioned, the figure also shows that there are moments in the formation of antibodies when the titer of antitumor antibodies considerably exceeds the titer of antibodies against antigens from normal spleen.

Our findings show that after late reimmunization with antigens from tumor tissue, the titer of antitumor antibodies is considerably higher, and their rate of production is more rapid than after primary immunization.

It was also found that, irrespective of the dose of antigens, in our experiments all the rabbits reacted to roughly the same degree after reimmunization.

The increase in the immunological reactivity of animals after late reimmunization with antigens from Ehrlich's mouse tumor (ascitic form), which we observed, corresponds to the results obtained in infectious immunology. In this connection it may be postulated that laws governing the relation between the immunological

reactivity of the animal and late reimmunization, which have been demonstrated in infectious immunology, are also of great importance in the immunization of animals with tumor material.

SUMMARY

This work deals with the effect of remote reimmunization of rabbits with antigens of the tissue of mice Ehrlich's tumor on the production of the antitumor antibodies. The data obtained demonstrate that following remote reimmunization with antigens of the tumor tissue the titer of the antitumor antibodies was considerably higher and the velocity of their production was greater that after primary immunization. After the second immunization all the rabbits reacted in about the same way, regardless of the antigen dose.

LITERATURE CITED

[1] G. V. Vygodchikov, Textbook on Work with Sera and Vaccines. Moscow, 1943 [In Russian].

[2] P. F. Zdrodovskii, The Problem of Reactivity in Infection and Immunity. Moscow, 1950 [In Russian].

[3] M. Raiskii, Khar'kovsk. Med. Zhur., 20, No. 8, 140 (1915).

[4] P. P. Filatov, Byull. Éksptl. Biol. i Med., No. 6, 95, (1959).•

• Original Russian pagination. See C. B. Translation.

EXPERIMENTAL BIOLOGY

THE PRECIPITATION REACTION BETWEEN THE FERTILIZIN OF THE OVA AND THE ANTIFERTILIZIN OF THE SPERMATOZOA IN POLYCHAETA OF THE WHITE SEA

L. L. Lishtvan

From the Laboratory of Immunology of Embryogenesis (Head—Candidate Med. Sci. O. E. Vyazov) of the Institute of Experimental Biology of the AMN SSSR (Director—Prof. I. N. Maiskii) and the White Sea Biological Station of Moscow State University (Director—N. A. Pertsov)

(Received April 3, 1959. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

The reaction of fertilization is known to possess high species and tissue specificity [5]. In 1919 Lillie [5] put forward a hypothesis according to which the first phase of fertilization—the attachment of the spermatozoon to the homologous ovum—follows a similar course to the antigen—antibody reaction. The role of antigen and corresponding antibody is played by substances connected with the surface of the gametes—the fertilization of the ovum [4] and the antifertilization of the spermatozoon. Many facts have now been collected which tend to show that fertilizin and antifertilizin, which take part in the combination of the gametes, are substances possessing mutually complementary physicochemical properties [8, 9]. The fact that the onset of development (activation) of the ovum is brought about largely by a reaction of antigen-antibody type suggests that immunological reaction play an important part in the course of ontogenesis [1].

Authors who have worked on the problem of the immunology of fertilization have studied mainly the biological role and the chemical nature of fertilizin and antifertilizin [11, 17]. The mechanism of interaction of these substances has not yet been fully studied. We therefore considered it important to investigate the behavior of solutions of fertilizin and antifertilizin by means of a classical immunological reaction—the precipitation reaction.

EXPERIMENTAL METHOD

In the work we used mature gametes of two species of Annelida—Nereis virens and Arenicola marina, belonging to two sub-classes of the class Polychaeta. A solution of fertilizin ("egg water") of N. virens was obtained by allowing a 20% suspension of ova in sea water to stand for 24 hours. After standing, the suspension was centrifuged at a speed of 3000 rpm for 10 minutes. In the reaction we used the transparent, rapidly gelatinizing supernatant fluid.

A solution of fertilizin from A. marina, the ova of which have not such a well-marked gelatinous membrane as the ova of N. virens, was obtained by allowing a 50% suspension of ova in sea water to stand, with periodic shaking, for 24 hours. The supernatant fluid obtained after centrifugation was transparent, colorless, and sometimes was not gelatinized.

Solutions of antifertilizin were prepared from the sperm of the two species of worms in the same way: a 20% suspension of spermatozoa in sea water was heated to 90° three times, after which it was centrifuged at 3000 rmp for 39 minutes. In the reaction we used the transparent supernatant fluid.

TABLE 1

Results of the Ring-Precipitation Test (a) and the Agar Gel Precipitation Test (b) between Fertilizin and Antifertilizin from N. Virens

a)

"Antigen" / "Antibody"	Antifertilizin of N. virens dilutions						
	1:1	1:3	1:10	1:50	1:100	1:500	1:1000
Fertilizin of N. virens (1:3)	+++	++	++	+	+	+	-

b)

"Antigen" / "Antibody"	Antifertilizin of N. virens
Fertilizin of N. virens	1 well-marked ring and 1 weak ring

In this and the following tables fertilizin is conventionally called "antibody" and antifertilizin "antigen"

TABLE 2

Results of the Ring-Precipitation Test Between Fertilizin of A. marina and Antifertilizins from Members of Different Types of Invertebrates

"Antigen" / "Antibody"	Antifertilizin of A. Marina	Antifertilizin of N. virens	Antifertilizin of O. Sarsi	Fluid from the cavity of S. Rustica (1:1)	Control-sea water
Fertilizin from A. marina (1:1)	+++	++	—	—	—
Control — sea water	—	—	—	—	

The ring-precipitation test and the agar precipitation test in capillary tubes, as modified by us, were used in the investigation.

TABLE 3

Results of the Ring-Precipitation Test between Fertilizin from N. virens and Antifertilizin from N. virens and A. marina

"Antigen" \ "Antibody"		Fertilizin from N. virens (1:1)
Antifertilizin from N. virens	1:1	+++
	1:3	++
	1:10	++
	1:50	+
	1:100	+
	1:500	+
	1:1000	–
Antifertilizin from A. marina	1:1	+
	1:3	+
	1:10	+
	1:50	+
	1:100	–
	1:500	–
	1:1000	–

TABLE 4

Ring-Precipitation Test between Fertilizin and Antifertilizin, and Blood of A. marina

"Antigen" \ "Antibody"		Fertilizin from N. virens (1:1)
Antifertilizin from A. marina	1:1	+
	1:3	+
	1:10	+
	1:50	+
	1:100	–
Blood from A. marina	1:1	–
	1:3	–
	1:10	–
	1:50	–
	1:100	–

EXPERIMENTAL RESULTS

The results obtained in the experiments are shown in Tables 1-4.

As will be apparent from the results shown in Table 1, fertilizin and antifertilizin react with each other in the ring precipitation test and in the agar gel precipitation test like typical antigen and corresponding antibody.

In order to study the type specificity of the precipitation reaction observed between solutions of fertilizin and antifertilizin, experiments were carried out in which these substances, obtained from members of different types of invertebrates, were combined: fertilizin from A. marina and antifertilizin from A. marina, N. virens (Annelida), Ophiura sarsi (Echinodermata) and fluid from the cavity of the ascidium Styela rustica (Chordata).

The results of this experiment are shown in Table 2.

It can be seen from Table 2 that positive results were obtained only in the reaction with "antigens" of the Annelida type. In all other cases the results were negative.

The experiments results thus showed that the fertilizin-antifertilizin reaction is characterized by type specificity.

It was interesting to find out whether this reaction showed even narrower specificity within the class Polychaeta. In an experiment, the results of which are shown in Table 3, we used the ring-precipitation test between fertilizin from N. virens and antifertilizin from N. virens and A. marina.

It is clear from Table 3 that antifertilizin from N. virens reacted in a dilution of 1:500 with fertilizin of the same species of animal. Antifertilizin from A. marina reacted with fertilizin from N. virens only in a dilution of 1:50. These findings show that the combination of fertilizin and antifertilizin on the gametes of these species possesses a relative species specificity in the precipitation reaction. At the same time, our experiments showed that cross fertilization does not occur between these two species. We never once observed the attachment of the spermatozoa of N. virens with the surface of the ova of A. marina.

The reaction between fertilizin and antifertilizin was thus more specific in those cases when these substances were combined with the surface of the gametes than when they were in solution. These results are in agreement with Tyler's views, based on the study of the agglutination reaction between the sperm of sea-urchins and a solution of fertilizin.

We also tested the tissue specificity of the fertilizin-antifertilizin reaction. We carried out ring-precipitation experiments between a solution of fertilizin from N. virens on the one hand and blood from A. marina on the other.

The results of these experiments are shown in Table 4.

It will be seen from the results in Table 4 that fertilizin from N. virens reacted only with antifertilizin from A. marina. The test with the blood from A. marina was negative. This indicates the tissue specificity of the fertilizin-antifertilizin reaction.

From the experiments described above it was possible to show directly that the reaction between solutions of fertilizin and antifertilizin proceeds along the lines of antigen-antibody reaction.

The reaction between solutions of fertilizin and antifertilizin is characterized by type- and tissue-specificity, as well as by relative species-specificity.

The results described, in our opinion, suggest that the primary immunological reactivity plays a definite role in the process of fertilization.

SUMMARY

The author studied the precipitation reaction between solutions of fertilizin of the ova and antifertilizin of the sperm in Nereis virens and Arenicola marina.

The reaction between fertilizin and antifertilizin solutions is characterized by a type-, tissue-, as well as a relative species-specificity. The reactions between these substances are more specific when they are connected with the gamete surface than when in solution.

LITERATURE CITED

[1] O. E. Vyazov, Uspekhi Sovremennoi Biol., 33, 1, 47 (1952).

[2] N. N. Zhukov-Verezhnikov, Uspekhi Sovremennoi Biol., 18, No. 1, 93 (1944).

[3] N. N. Zhukov-Verezhnikov, Proceedings of the Eighth All-Union Congress of Hygienists, Epidemiologists, Microbiologists and Specialists in Infectious Diseases, 2, P. 55. Moscow, 1956 [In Russian].

[4] F. R. Lillie, Science, 1912, v. 36, p. 527.

[5] F. R. Lillie, Problems of Fertilization. Chicago, 1949.

[6] J. Ronnström, in book: Sovremennye Problemy Tsitiologii (Moscow, 1955) p. 253.

[7] A. Tyler, Proc. Nat. Acad. Sc., 1939, v. 25, p. 317.

[8] A. Tyler, Some Problems in Present-Day Embryophysiology, p. 53. Moscow, 1951 [Russian translation].

[9] A. Tyler, Analysis of Development. Philadelphia – London, 1955, p. 170.

[10] A. Tyler, Exper. Cell. Res., 1956, v. 10, p. 377.

THE MECHANISM OF THE DIURNAL PERIODICITY OF MITOSIS

I. A. Alov

From the Department of Histology (Head – Prof. I. A. Alov) of the Khabarovsk Medical Institute (Director – Docent S. K. Nechepaev)

(Received February 12, 1959. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The changes in mitotic activity in the course of the 24 hours is a particular manifestation of the diurnal periodicity of various physiological processes in the body. The diurnal mitotic regime has been studied in detail in plants, in the protozoa, and in various organs of animals and man. Despite the large number of investigations on the subject of the diurnal periodicity of mitosis in normal and pathological conditions [4, etc], the mechanism of these processes remains far from clear.

From a number of observations it may be deduced that the diurnal variations in mitotic activity are connected with the variations in light [15, 16, 19], with changes in the temperature or with the rhythm of functional activity of the body [9, 10, 15]. In the present work an attempt was made to discover experimentally the significance of these factors in bringing about the diurnal periodicity of mitosis in the animal body.

TABLE 1

Changes in Mitotic Activity in Response to Disorganization of the Diurnal Alternation of Light and Darkness •

Experimental conditions	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)			
			cornea	skin	tongue	esophagus
Natural alternation of light and darkness	6	Morning	486±24 K – 1,3	22±2 K – 2,6	114±14 K – 1,5	70±8 K – 1,3
	6	Evening	105±23 K – 1,9	9,6±2,5 K – 3,4	43,6±3,6 K – 1,0	6,0±1,4 K – 1,4
Disorganized alternation of light and darkness	6	Morning	119±14 K – 2,0	5,8±1,5 K – 2,0	40±4 K – 2,7	5,1±1,0 K – 1,6
	6	Evening	482±30 K – 1,9	20,6±1,9 K – 2,2	147±20 K – 1,3	66±16 K – 1,7

• In all the groups P = 0.001-0.002.

TABLE 2

Diurnal Variations in Mitosis in Rats after Adrenalectomy

Group of animals	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)			
			cornea	skin	tongue	esophagus
Control	6	Morning	156±13 K—2.0	136±11 K—0.9	32±7.6 K—1.3	18±3.1 K—1.5
	6	Evening	11,4±2 K—2.5	72±15 K—1.5	2±0,9 K—3.0	8.5±2.4 K—1.4
			P<0,001	P=0,004	P=0,002	P=0,01
After thyroidectomy	6	Morning	105±21 K—2.2	86±15 K—1.0	29±4,1 K—1.2	20±4.9 K—1.1
	6	Evening	8±2.6 K—2.6	96±26 K—1.6	18,2+3.2 K—1.9	12±3.4 K—1.4
			P=0,001	P=0.38	P=0.04	P=0.11

TABLE 3

Diurnal Variations in Mitosis in Rats after Thyroidectomy

Group of animals	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)			
			intestine	tongue	cornea	skin
"Night"	7	Morning	412±21 K—1.4	146±15 K—1.3	290±29 K—1.5	23±6 K—2.1
	7	Evening	252±13 K—2.2	24±3.5 K—2.0	172±71 K—1.3	7,6±2 K—1.0
			P<0.001	P<0.001	P=0.08	P=0,018
"Day"	6	Morning	314±19 K—1.8	48±3.7 K—1,1	286±36 K—1.6	18±1.6 K—2,0
	6	Evening	420±22 K—1.6	78±6 K—1.9	91±23 K—1.6	11±0.7 K—2.2
			P=0.01	P=0,002	P=0,001	P<0,001

EXPERIMENTAL METHOD

Experiments were carried out on white mice and rats (males) aged 2-3 months. The mitotic activity was determined in the morning (8A.M.) and evening (8 P.M.) in the epithelium of the cornea, skin, tongue, esophagus and intestine and in the lymphocytes of the thymus gland. The magnitude of the mitotic activity was judged by the number of dividing cells per constant area (1.65 mm^2), by the percentages of the individual phases of mitosis and by the phase coefficient.

TABLE 4

Changes in the Mitotic Activity during Organization and Disorganization of the Feeding Program

Group of animals	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)				
			cornea	skin	tongue	esophagus	thymus
Control	6	Morning	318±34 K—2.4	37±4.2 K—3.1	91±13 K—0.8	36±3.9 K—1.9	117±16 K—3.0
	6	Evening	93±12 K—2.6	10±2.6 K—3.0	60±11 K—0.9	14±5.5 K—1.0	68±19 K—2.2
			P < 0.001	P < 0.001	P—0.06	P—0.003	P—0.04
After adrenalectomy	6	Morning	260±35 K—2.9	20±3.3 K—1.5	107±8.9 K—1.1	40±6.3 K—1.2	105±12 K—2.8
	6	Evening	115±25 K—2.2	12±1.3 K—2.6	70±11 K—1.2	12±2.5 K—1.0	66±14 K—2.7
			P—0.004	P—0.02	P—0.05	P—0.001	P—0.03

In the first series of experiments we studied the diurnal variations in mitoses when the normal periodicity of light was disturbed during the 24 hours. The groups of experimental mice were kept in artificial illumination during the night (9P.M. to 7A. M.) and during the daytime (7 A. M. to 9 P. M.) they were kept in total darkness. Control animals were kept in ordinary conditions of natural alternation of day and night. The experiment was continued for 40 days, for the main guarantee of success in the experiment was its duration.

In a second series of experiments we studied the diurnal variations of mitoses in white rats 8 days after bilateral adrenalectomy.

In the third series of experiments rats were subjected to the operation of thyroidectomy. The mitotic activity was determined in the morning and evening of the 9th day after the operation.

In the fourth series of experiments we studied the mitotic activity when the digestive activity was disorganized. The first group of mice in this series (the "night" group) received food only during the night (from 8 p.m. to 8 a.m.), and the food was withdrawn during the day. The animals of the second group (the "day" group) received food only during the daytime (from 8 A.M. to 8 P.M.) and food was withdrawn during the night. Both groups of mice were kept under identical conditions of illumination (a semi-darkened room). The experiment lasted 2 months. The mice were sacrificed in the morning (7:30 A.M.) and evening (7:30 P.M.). Since in this series we assumed that the general physiological activity of the animal was disorganized as well as the digestive activity, at the beginning of the experiment we periodically introduced a female into the cage with the male mice for 2.3 days: at night with the "night" group of animals and during the daytime with the "day" group. In all the experiments we studied the RNA and DNA content of the tissues as well as determining the mitotic activity. The material was fixed with Helly's mixture. Sections were stained with methyl green and pyronine by Unna's method (control sections being treated with ribonuclease) and by Feulgen's method.

EXPERIMENTAL RESULTS

The results of the first series of experiments showed that disorganization of the alternation of day and night led to complete disorganization of the diurnal periodicity of mitotic activity (Table 1). In animals in natural conditions of diurnal periodicity of light and darkness, the maximum values of mitotic activity in the epithelium of the cornea, skin, tongue and esophagus occurred in the morning, and the minimum values in the evening. In the

animals of the experimental group (illumination at night and darkness by day) the diurnal rhythm of mitosis was completely disorganized. Maximum values for the mitotic activity were observed in the evening and minimum values in the morning. The results of this series of experiments thus showed that one of the important factors determining the diurnal rhythm of mitosis is the periodic alternation of day and night, of light and darkness.

A number of workers have described the stimulation by light of the hypophysis, the sex glands, the thyroid gland and the adrenals, and the changes in the activity of these organs on account of diurnal alternations of light and darkness [3, 8, 9]. The endocrine organs also play an important role in the regulation of cell division [1, etc.]. It has also been found that stimulation of mitoic activity by the supplementary illumination of animals is effected through the thyroid gland [7]. These observations suggest that the effect of the diurnal alternation of light and darkness on the mitotic regime is connected with changes in the activity of the endocrine organs.

Experiments were accordingly carried out in which the diurnal variations in mitosis were studied in animals after preliminary extirpation of the adrenals and thyroid gland. The results of these experiments showed that after adrenalectomy (Table 2) and after thyroidectomy (Table 3) the diurnal variations in mitosis in the morning and evening were maintained. The scale of variation of mitotic activity in the morning and evening was, however, slightly reduced in some cases. This was noted in the epithelium of the cornea and skin after adrenalectomy and in the epithelium of the tongue, esophagus and skin after thyroidectomy. The fluctuations in mitosis in the epithelium of the tongue between morning and evening were completely obliterated. From the results of these experiments it may be postulated that the adrenals and the thyroid gland are of some importance in effecting the diurnal rhythm of mitosis in animals. The role of these organs, however, is evidently not a decisive one, for after their extirpation the diurnal fluctuations in the number of mitoses are maintained. This appears all the more likely because a diurnal periodicity of mitosis is present in protozoa and plants, and the diurnal mitotic regime in different organs of animals is not identical.

In the experiments in which the diurnal alternation of light and darkness was disturbed, attention was drawn to the fact that the changes in the alternation of day and night led at the same time to a complete disorganization of the rhythm of functional activity of the animal. In the mice illuminated during the night (the "night" group of animals) the maximum level of functional activity was shifted to the daytime. These observations suggested that the effect of alternation of day and night on the mitotic regime was brought about primarily by an alternation in the functional rhythm of the different organs. The digestive organs were selected as a test object.

Preliminary experiments (on 41 animals) showed that the intestinal epithelium evidently possesses a different diurnal rhythm of periodicity of mitosis from that of other organs [15]. In any case, we observed no essential difference between the mitotic activity in the morning and evening (morning 326 ± 17, evening 350 ± 11).

The maximum digestive activity of laboratory mice is known to occur during the night, although they periodically ingest food during the day. If the diurnal rhythm of the mitosis were connected with the functional activity of an organ, then it might have been expected that with a modification or complete disorganization of the feeding program, the diurnal rhythm of mitosis would also be changed. A third series of experiments was accordingly carried out, in which some mice (the "night" group) received food at night only, and the others (the "day" group) received food only by day. Although the experiment lasted for two months, we were unable to bring about complete disorganization of the general functional activity in this way. The change in the digestive activity was the result of the experimental environment itself. The results of these experiments (Table 4) showed that feeding animals only at night leads to the appearance of obvious diurnal variations in mitosis in the intestinal epithelium.

With disorganization of the feeding program (in the daytime) the diurnal variations in mitosis became inverse in nature: the higher the level of mitotic activity observed in the evening, the lower the values in the morning. The diurnal variations in mitosis in the epithelium of the tongue were disorganized at the same time. The difference between the mitotic activity in the morning and evening in the "day" group of animals was less than that in those animals in which only the feeding program was disturbed from its normal pattern. The diurnal variations in mitosis in the epithelium of the cornea and skin were essentially unchanged. In two experiments, however, unusually high levels of mitotic activity were observed in the "night" group of animals in the evening. It is possible that, had the experiments been continued longer, changes would have occurred in the general functional activity of the animal and in the mitotic regime in organs unrelated to the digestive system. The rhythm of functional activity of the cells determines the mitotic regime, probably by modifying the metabolic processes with which mitotic division is associated.

From a number of observations, including those in our own laboratory [2, 5], it may be considered that one of the essential links in the biochemical mechanism of mitosis is the nucleoprotein metabolism. From this it may be deduced that diurnal variations will take place in the RNA and DNA contents, corresponding to the variations in mitotic activity. Such changes have been described in the liver cells [14, 6]. Our observations were conducted on the epithelium of the cornea, tongue, intestine and liver and on the lymphocytes of the thymus gland in rats and mice (A. A. Zhirnova). The results of these experiments showed that, corresponding to the diurnal variations in mitotic activity, obvious diurnal variations in the RNA and DNA content of the cells were observed. In the epithelium of the cornea, tongue and liver, and in the lymphocytes a higher content of nucleic acid was found in the morning and a lower content in the evening. The RNA and DNA content of the intestinal epithelium (and the mitotic activity correspondingly) showed no essential difference between morning and evening. When the diurnal alternation of light and darkness was disturbed, obvious changes took place in the nucleic acid content of the cells. When the animals were illuminated at night, the maximum content of RNA and DNA was shifted from the morning to the evening.

The experimental results thus show that the diurnal periodicity of mitosis is connected with various factors. The diurnal alternation of light and darkness, the pattern of functional activity and changes in metabolic reactions are all links in the complex chain of processes responsible for the diurnal rhythm of mitosis. The final links of this chain must evidently be sought in the changes in the nuclein metabolism in the course of the day.

SUMMARY

Inversion of the alternation of light and darkness during the 24-hour period resulted in a complete inversion of the diurnal rhythm of mitotic activity in the epithelium of the skin, cornea, tongue and esophagus. After removal of the adrenal or thyroid glands the diurnal fluctuations of the number of mitoses were preserved, but their range in the morning and evening hours was somewhat reduced. Diurnal fluctuations of mitotic activity, absent in control animals, were noted in mice fed only at night. When mice were fed only in the morning the diurnal fluctuations were changed in the intestinal and tongue epithelium. The diurnal fluctuation of the RNA and DNA content noted in the cells of different organs corresponded to the mitotic rhythm. When the animals were illuminated at night the time of the maximum nucleic acid content changed from the morning to the evening.

LITERATURE CITED

[1] I. A. Alov, Byull. Éksptl. Biol. i Med., 39, No. 11, 63 (1955); No. 12, 47 (1955).

[2] I. A. Alov, Trudy Khabarovsk. Med. Inst., 15, 31 (1957).

[3] I. A. Alov, Trudy Khabarovsk. Med. Inst., 16, 21 (1958).

[4] I. A. Alov, Voprosy Onkol., 5, No. 1, 7 (1959).

[5] A. A. Voitkevich, Izvest. Akad. Neuk SSSR. Ser. Biol., No. 4, 385 (1945).

[6] M. T. Gololobova, Byull. Éksptl. Biol. i Med., 46, No. 9, 118 (1958).•

[7] L. M. Ermolenko, Byull. Éksptl. Biol. i Med., 45, No. 2, 102 (1958).•

[8] L. D. Liozner, Z. A. Ryabinina and V. F. Sidorova, Proceedings of the Sixth All-Union Congress of Anatomists, Histologists and Embryologists, p. 333. Khar'kov, 1958 [In Russian].

[9] I. E. Epova, Byull. Éksptl. Biol. i Med., 43, No. 2, 80 (1957).•

[10] I. A. Éskin and G. M. Vidavskaya, Problemy Éndokrinol. i Gormonoterap., 2, No. 1, 82 (1956).

[11] T. Bissonete, Quart. rev. biol., 1936, v. 11, p. 371.

[12] C. M. Blumenfeld, Arch. Pathol., 1942, v. 33, p. 770; 1944, v.38, p. 321.

[13] W. S. Bullough, Proc. Roy. Soc. Lond., Ser. B., 1948, v. 135, p. 212; Exper. Biol., 1949, v. 26, p. 76.

[14] A. Carleton, J. Anat. Lond., 1934, v. 68, p. 251.

[15] F. Halberg, J. J. Bilttner and D. Smith, Ztschr. Vitamin., Hormon and Fermentforsch, 1957, Bd. 9, S.69.

• Original Russian pagination. See C. B. Translation.

[16] F. Halberg, C. P. Barnum and R. H. Silber and others, Proc. Soc. Exper. Biol et Med., 1958, v. 97, p. 987.

[17] H. Klein and H. Geisel, Klin. Wschr., 1947, v. 24-25, p. 662.

[18] M. Meyer, Ztschr. Zellforschr., 1954, Bd. 40, S. 228.

MORPHOLOGY AND PATHOMORPHOLOGY

MORPHOLOGICAL CHANGES IN THE SKIN IN RESPONSE TO EXTERNAL α-IRRADIATION

F. L. Leites

From the Radiological Laboratory (Head—Prof. E. S. Shchepot'eva) of the State Research Institute of Spa Therapy and Physiotherapy (Director—G. N. Pospelova), Moscow

(Received February 24, 1959. Presented by Active Member AMN SSSR I. G. Rufanov)

The biological action of α-rays and, in particular, the morphological changes resulting from external α-irradiation have been inadequately studied. For this reason it is not yet clear whether external α-irradiation is harmful, and if it is, how its action differs qualitatively from that of other types of ionizing radiation. There are reports in the literature [2, 4, 14] of the extremely limited harmful action of α-irradiation from external sources, such as: "α-Rays, when acting externally, may have a harmful effect only in exceptional cases, for example when they act on the mucous membranes" [2]. In contrast to this view, there are reports [6] of the development of deep necrosis and ulceration after irradiation of the skin of rabbits with α-rays from polonium.

In view of the scanty and controversial information on the pathological changes in the tissues in response to the action of external α-irradiation, we considered it of interest to investigate these changes over a period of time, varying the doses of radiation absorbed.

EXPERIMENTAL METHOD

Experiments were carried out on 35 rabbits weighing 2.0-2.5 kg, to the shaved dorsal skin of which were applied radioactive binders containing fission products of thoron (thorium B, thorium C, thorium C_I and thorium C_{II}). These radioactive isotopes emit α-, β- and γ-rays, but it can be considered that their action is due mainly to α-rays which account for more than 90% of the radiation energy absorbed by the tissues [1, 15, 16, 19]. The activity of the applicator was 4-5 $\mu C/cm^2$ in the first series of experiments, 0.3 $\mu C/cm^2$ in the 2nd series and 0.06 $\mu C/cm^2$ in the 3rd series. The applicator, 8 × 8 cm in size, was placed in position for a single period of 20 hours or 5-6 times at intervals of 1 week. In the same conditions a binder free from radioactivity was applied to 5 rabbits. The animals were sacrificed at various periods of time (from 1 day to 8 months from the beginning of the experiment) with subsequent morphological examination of the skin and inspection of the internal organs. In some animals biopsy of the skin of irradiated and unirradiated symmetrically opposite areas (as controls) was performed.

EXPERIMENTAL RESULTS

Before estimating the morphological changes after irradiation it had to be ensured that this was external, i.e., that the radioactive isotopes incorporated in the applicator had not penetrated into the skin tissue. For this purpose biopsy specimens of skin taken from the experimental rabbits immediately after irradiation at intervals of 5, 24, 48, 72 and 96 hours, were investigated by the histoautoradiographic method of mounting [7, 20].

Microscopic examination of the histoautoradiograms (11 experiments in all, and 15-20 sections of skin investigated in each case) showed that the characteristic "stars" of the isotope emitting α-rays were present on the

Fig. 1. Changes in the skin of a rabbit three days after a single exposure to α-rays. Gross thinning of the epidermis, with gigantism and a transverse disposition of the nuclei in the cells which are left. Ocular 15×, objective 40×.

surface of the skin only, which was evidence of the transfer of a known quantity of radioactive material from the applicator to the skin. No radioactive material was found in the thickness of the epidermis and dermis. The largest quantity of isotopes on the surface of the skin was found immediately after removal of the applicator. Later on, on account of the rapid fission of the isotopes (maximum half-life period about 11 hours), their quantity progressively fell, so that after 3-4 days the skin was practically free from radioactivity. Judging by the data from histoautoradiography, therefore the radioactive substances tested did not penetrate into the skin or were practically completely disintegrated before they could possibly penetrate the skin. For this reason the action of the radioactive applicators on the body was entirely due to external irradiation. The length of penetration into the tissues by α-particles emitted by the fission products of thoron, from measurements with the ocular micrometer, from our findings was 50-60 μ which is only very slightly more than the thickness of the epidermis in the dorsal region of the rabbit's skin.

During the period of irradiation and for a year after its conclusion, the general condition of the animals appeared normal. Histological investigation of the internal organs also revealed no pathological changes. Macroscopic changes in the skin were manifest in the form of hyperemia, pigmentation, and desquamation, varying in accordance with the size of the dose absorbed. With whatever dose was used, the microscopic changes were mainly found in the epidermis. In the first series of experiments, for instance, immediately after removal of the applicator, individual cells of the basal layer of the epidermis were seen with swollen and unusually light nuclei and

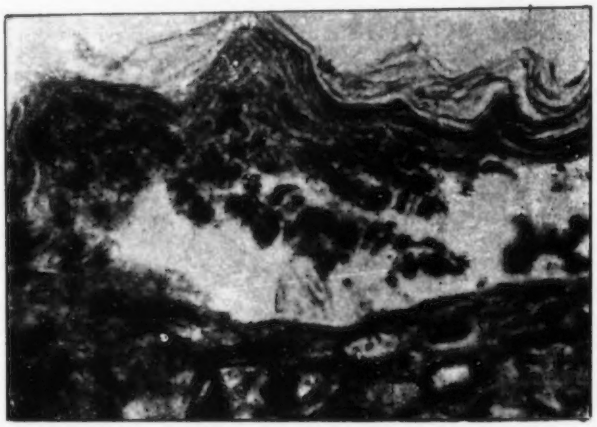


Fig. 2. Changes in the skin of a rabbit after repeated exposure to α-rays. Formation of a vesicle in the epidermis. Severe dystrophy of the epithelium at the base of the vesicle. Objective 40×, ocular 15 ×.

large, eccentrically situated nucleoli within them, arranged against a background of the main mass of unchanged cells. The number of these cells later increased, and this was accompanied by abnormalities of differentiation of the cells of the epidermis and vacuolation of the cells of the stratum spinosum. Three and 4 days after irradia-

Fig. 3. Imperfect regeneration of the epidermis of a rabbit. Obliteration of the cell borders and the formation of ridges. Ocular 10×, objective 40×.

tion, cells with giant, deformed nuclei, arranged parallel to the skin surface, and also isolated small pycnotic cell. were observed in the basal layer (Fig. 1). Staining by Feulgen's method at this period showed a sharp fall in the content of desoxyribonucleic acid in the nuclei of the swollen and giant cells and an increase in the content of DNA in the pycnotic cells.

One week after irradiation, the epidermis presented a picture of severe disturbance of differentiation, with pycnosis and karyocytosis of many cells of the basal layer, severe hypertrophy and deformation of the nuclei in the cells which were preserved and with the onset of desquamation of the stratum corneum together with the underlying dead epithelium. Pigment formation began at this period, in the form of the appearance of fine granules of melanin in the perinuclear zones and alone the cells borders in the basal layer. The degree of the dystrophic changes in the epidermis and of deposition of pigment were unevenly expressed over the skin surface. This "spotted" character of the changes was evidently connected with distinctive properties of this source of radiation — the uneven distribution of radioactive material on the surface of the applicator and the criss-cross structure of the applicator itself, which was made of gauze.

Two weeks after a single exposure to irradiation, even more marked dystrophic changes were present in the epidermis, which in some areas was thinned to three, two or even one layer of cells, with huge polymorphic nuclei, situated in various positions. An abundance of pigment was present in the cytoplasm of three cells. In these areas the stratum corneum was usually absent, but sometimes it was observed to be desquamated together with the underlying of dead epithelium, in which the outlines of giant pycnotic nuclei could vaguely be distinguished. Three and four weeks after a single exposure to irradiation, signs of progressive regeneration were observed in the skin, with proliferation of the epidermis in its basal layer and desquamation of the superficial layer of abnormal epithelium.

After the course of irradiation was completed, the changes in the epidermis in the 1st series of experiments were more noticeable. The thinning and the deposition of abundant pigment were almost diffuse. Desquamation of the stratum corneum together with the underlying necrotic epidermis was accompanied by penetration of edema fluid into this layer, together with a few leucocytes, and the formation of vesicles (Fig. 2). The base of the vesicles was often formed by dermis, in which there was obvious leucocytic infiltration. In the areas of the hair follicles next to the epidermis, dystrophy of the epithelium was present, and here and there the hair was shed and the follicles dilated and deformed. One week after the course of irradiation, the dystrophic changes in the epidermis were accompanied by imperfect regeneration of that structure, with obliteration of the cell borders and the basal membrane and the formation of characteristic ridges (Fig 3). Only two weeks after the course of irradiation, however, the regeneration of the epidermis was normal again; proliferation of an epidermis of typical structure was observed, slightly thinner than in the control animals. Areas of severe dystrophic changes could still be seen at this period against the background of intensive regeneration.

After a single or multiple exposure to irradiation, the dermis in the 1st series of experiments in the course of the first week showed a moderate capillary hyperemia in the stratum papillare against a background of very

slight edema, palisading of the leucocytes in the small vessels and slight perivascular leucocytic infiltration. To this was subsequently added a slight, diffuse and nodular (beneath the epidermis) histiocytic proliferation, accumulation of pigment in the histiocytes of the dermis, and isolated giant histiocytes. No changes were found in the argyrophilic collagen and elastic fibers of the skin after a single exposure to the radiation; after repeated exposure, at the end of the course, impregnation by the Tibor-Pape* method showed a transient coarsening of the subepidermal argyrophilic membrane and swelling of the collagen fibers in the stratum papillare. Restoration of the skin structure after a single exposure to irradiation in the 1st series of experiments began in earnest after one month; after repeated irradiation – after 3-4 months, although at this period too, traces of its effect could still be seen in the form of the presence of pigment in the dermis and small areas of epidermis in which the basal layer was irregular in its structure.

After the absorption of smaller doses of radiation (2nd and 3rd series), dystrophic and necrobiotic changes were also observed in the epidermis, although these developed much more slowly. In the 2nd series, for instance, after 3-4 exposures, isolated hypertrophied cells with vacuolated cytoplasm and eccentrically situated nuclei were found in the epidermis. To these were subsequently added, in turn, maldifferentiation and polymorphism of the epidermis, gigantism and transverse disposition of the nuclei, and thinning of the epidermal layer to 1-2 rows of cells. After the conclusion of irradiation in the 2nd series of experiments, the changes thus resembled those which took place during the 2nd week of the experiment in the 1st series. In the 3rd series of experiments, it was only at the end of the course of irradiation that foci of dystrophy and necrobiosis of the epidermis were seen, with thinning of the epidermis in these areas and excessive accumulation of melanin in the cells. Changes in the dermis in the 2nd series were shown merely by a transient dilatation of the small vessels in the stratum papillare and by slight perivascular leucocytic infiltration. In the 3rd series no morphological changes were observed in the dermis.

Consequently, external α-irradiation, although leading to obvious dystrophic changes in the epidermis, was not accompanied by any significant involvement of the dermis, and for this reason did not produce sclerosis nor atrophy of the skin. If we compare these changes with those described after the action of x-rays [5, 9, 10, 21, 22] and β-rays [11, 12, 17, 18, 23], we can observe that doses of x-rays and β-rays which led to such marked changes in the epidermis inevitably caused in addition a severe inflammatory reaction in the dermis, with (especially after the action of x-rays) subsequent atrophy of the skin appendages and sclerosis of the dermis. The distinction which we found in our experiments between the degree of the significant changes in the epidermis and the slight changes in the dermis is very probably connected with the physical properties of this radiation, and especially with the fact that α-particles, which posses a high ionization density (and, hence, high biological effectiveness) have a low penetrating power, so that the bulk of the α-particles are absorbed in the epidermis and do not enter the connective tissue layer of the skin.

The absence of any severe inflammatory reaction in the dermis after external α-irradiation also accounts for the fact that, in our experiments, no atypical proliferation of the epidermis and follicular epithelium took place in the later stages of the experiment, with the development in isolated cases of carcinoma of the skin, as is observed after β-irradiation [17]. This is understandable in the light of the work of V. G. Garshin [3], who showed conclusively that proliferative processes in the epithelium are largely dependent on the degree of the inflammatory reaction of the stroma.

As has already been pointed out, the skin changes after α-irradiation are completely reversible. The active course of the regenerative processes in our experiments was undoubtedly due to the superficial localization of the changes and also to the unevenness, the "spotted" nature of the lesions in the epidermis, so that areas which were only slightly affected acted as sources of regeneration. These findings are in agreement with the statement by G. S. Strelin [13] that when a source of radiation is in the form of grid, the areas which are least exposed to irradiation have a stimulating effect on the regeneration of the areas of skin which absorb a larger dose of radiation.

We also studied the morphological changes in the nerve elements of the skin after external α-irradiation. For this purpose, biopsy specimens of skin of experimental and control animals were stained for myelin by Spielmeyer's method and impregnated with silver by Campos' method. It was found that, corresponding to the slight inflammatory and dystrophic changes in the dermis, the nerve trunks of the stratum reticulare and the stratum papillare, as well as the receptors of the sebaceous glands and hair follicles situated in these layers, underwent no

* Name not verified.

morphological changes. So far as the palisade-like receptors terminating in the epidermis are concerned, when their terminal ramifications were intact, the prereceptor fibers at the height of the skin changes in the 1st and 2nd series of experiments showed increased argentophilia and irregular thickenings and spiking of their contours. These changes in the receptors of the epidermis disappeared 1-2 months after the cessation of irradiation, i.e., they were reversible. The neurohistological examination of the skin thus also emphasized the superficial localization of the pathological changes during external α-irradiation.

SUMMARY

The author studied the changes occurring in the skin of the rabbit in external alpha-irradiation. The latter has a pronounced injurious effect, the morphological manifestations of which are analogous to those observed in the action of other types of ionizing radiation. Besides this, the reaction of the alpha-irradiated tissue has a specific feature in the form of a sharp contrast between the degree of affection of the superficial and deep layers. The injury mostly involves the epidermis. This is due to the physical properties of alpha-radiation.

LITERATURE CITED

[1] S. N. Ardashnikov, F. L. Leites and M. L. Rait, Vestnik Dermatol. i. Venerol., No. 1, (1960).

[2] V. Bochkarev, I. Keirim-Markus and M. L'vova, Measurements of the Activity of Sources of Beta-and Gamma-Irradiation. Moscow, 1953 [In Russian].

[3] V. G. Garshin, Inflammatory Proliferative Lesions of the Epithelium and Their Biological Importance in Relation to the Problem of Cancer. Moscow-Leningrad, 1939 [In Russian].

[4] N. G. Gusev, A Reference Book on Radioactive Radiations and Protection. Moscow 1956 [In Russian].

[5] V. I. Zarubin, Russk. Arkh. Patol., 6, 535 (1898).

[6] N. I. Ivanov, Transactions of the Central Institute of Roentgenology and Radiology, 5, pp. 194-202. Moscow, 1941 [In Russian].

[7] G. S. Lykova et al., Aids to Histoautoradiography. Leningrad, 1957 [In Russian].

[8] V. S. Minasov, Vestnik Venerol. i. Dermatol., No. 1, 16-20 (1953).

[9] B. N. Mogil'nitskii, Transactions of the Central Research Institute of Roentgenology and Radiology, Vol. 3, pp. 107-109. Moscow, 1940 [In Russian].

[10] M. I. Nemenov, Roentgenology, Vol. 1, pp. 237-249. Moscow-Leningrad, 1926 [In Russian].

[11] A. Ya. Prokopchuk, Theses of Sectional Speeches at the All-Union Conference on Medical Radiology. Clinical Section, p. 120. Moscow, 1956 [In Russian].

[12] V. A. Prokopchuk, Proceedings of the First Belorussian Conference of Anatomists, Histologists, Embryologists and Topographical Anatomists, p. 238. Minsk, 1957 [In Russian].

[13] G. S. Strelin et al., Doklady Akad. Nauk SSSR, 75, No. 3, 465-468 (1950).

[14] Reference Book on Radiometry, p. 177. Moscow, 1957 [In Russian].

[15] A. F. Tret'yakov, A New Form of α-Ray Therapy—the Radioactive Binder—and its Use in the Treatment of Patients with Radiculoneuritis. Author's Summary of Dissertation. Moscow, 1957 [In Russian].

[16] A. F. Tret'yakov, Zhur. Nevropatol. i Psikhiat., 55, No. 9, 689 (1955).

[17] L. V. Funshtein et al., The Use of Radioactive Phosphorus in the Treatment of Skin Diseases, p. 46. Moscow, 1955 [In Russian].

[18] A. M. Kharchenko and I. N. Vinokurov, Vestnik Venerol. i Dermatol., No. 5, 14 (1955).

[19] E. S. Shchepot'eva, Med. Radiol., No. 3, 62 (1957).

[20] E. V. Erleksova, Radioactive Isotopes in Medicine and Biology, p. 136. Moscow, 1955 [In Russian].

[21] G. Miescher, Strahlentherapie, 1927, v. 27, p. 257.

[22] G. A. Rost, Ibid., 1915, Bd. 6, S. 69.

[23] R. E. Zirkle, Biological Effects of External Beta Radiation, New York, 1951.

METHODS

A PNEUMOELECTRIC CONVERTER FOR PHYSIOLOGICAL RESEARCH

V. K. Sel'tser

From the Institute of Radiation Hygiene of the Ministry of Health of the RSFSR (Director—Corresponding Member AMN SSSR N. F. Galanin), Leningrad

(Received July 2, 1959. Presented by Active Member AMN SSSR V. V. Parin)

We propose a simple, inexpensive pneumoelectric converter, within the means of any laboratory, which provides for recording with magnetoelectric and electromagnetic loops, or for projection onto the screen of a cathode oscillograph a number of physiological functions (respiration, the pulsation of peripheral vessels, the plethysmogram and so on) in the form of curves of the usual shape. In order to obtain photographic records of

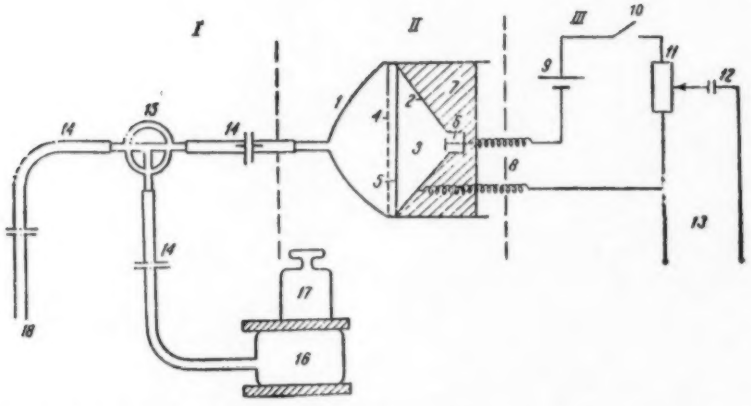


Fig. 1. General scheme of a simple pneumoelectric converter. I) Pneumatic transmission system with supplementary reservoir; II) pneumoelectric converter; III) scheme of feeding the converter and connecting the recording apparatus. 1) glass funnel; 2) body of microphone; 3) microphone chamber, filled withpowdered carbon; 4) perforated metal lid, protecting the diaphragm; 5) metal operating sensitive diaphragm; 6) fixed electrode of microphone with screw; 7) airtight packing; 8) lead from source of current; 9) source of current for microphone; 10) switch; 11) potentiometer (1.5 kohm); 12) condenser (3 μf); 13) lead to recording apparatus (amplifier and loop oscillograph or cathode oscillograph, or electrocardiograph, etc.); 14) rubber connecting tube; 15) 3-way cock; 16) supplementary reservoir beneath a weight; 17) weight, ensuring constant excess pressure in transmission system; 18) point of connection of pneumatic or hydraulic pick-up, sensitive to mechanical changes.

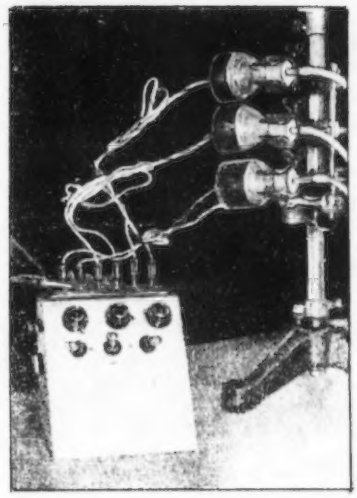


Fig. 2. Assembly containing three simple pneumoelectric converters. On the right – universal stand to which three converters are securely fixed. On the left – box in which is mounted a control panel and source of current for the three converters; on the top of the box are terminals for connection to the converters and the recording apparatus; the front of the box houses the knobs for the three potentiometers and three switches.

these phenomena with loop oscillographs, it is necessary to have a very simple amplifier, providing an amplification of not less than 500 of the signal received from the converter. In order to project the curves onto the screen of the tube of a cathode oscillograph, or to record them on an electrocardiograph, no additional amplification of the signal supplied by the converter is required.

The main operating part of the converter is a commercial model MK-10 carbon button microphone. The microphone is placed in a glass funnel with the diaphragm facing the narrow neck of the funnel (Fig. 1).

The wide part of the funnel is hermetically sealed with paraffin wax, Mendeleev's putty or other insulating material. Two leads pass through the sealing material, one of which is connected to the body of the microphone and the other to the screw of the fixed electrode. The lead to the microphone supplies current from any form of constant current source. We use a low tension battery 1.58–SNMTs-2.5 with an EMF of 1.65v.

The principle of the converter is illustrated in Fig. 1. The potentiometer (1.5 kohm) regulates the variations in current taken from the contacts of the converter, and thus serves to control the sensitivity of the system. The condenser (3 μf) protects the recording device from direct current. The parameters of the components listed above – the potentiometer, the source of current and the condenser – may be varied. The choice of parameters is determined by the sensitivity of the loops in use, the coefficient of amplification of the amplifier, and the character of the phenomena investigated.

The scheme illustrated in Fig. 1 ensures the constant high quality of work of the converter. Because of the special constructional features of the button microphone, the converter possesses high sensitivity and permits the recording of mechanical signals of a strength equivalent to fractions of a microwatt, but at the same time it is suitable for the recording of signals of considerable strength. The frequency band transformed by the converter is very broad and extends from tenths of a cps to the upper limit of audible frequencies. The hermetic transmission system reliably protects the converter from incidental noise. In order to ensure constant conditions of recording and comparable curves from different experiments, it is essential to maintain a constant excess pressure in the sensitive pick-up device and in the pneumatic transmission system, and this is done by the introduction of a 3-way cock and a supplementary reservoir, on which is a weight, into the system (see Fig. 1). The position shown in Fig. 1 is that of equilibrium of pressure. At the time of recording, the supplementary reservoir is disconnected by turning the cock. The magnitude of the excess pressure is determined by the object to be investigated. When recording the carotid pulse and respiration in rabbits, we created an excess pressure in the system of between 50 and 100 mm of water. A very important condition for the stable working of the converter is the airtightness of the whole pick-up and transmission system. It is imperative that the funnel with the microphone should be securely fixed to some form of stand and protected from extraneous vibration, which can be done satisfactorily by resting the feet of the stand on a sheet of sponge rubber. When the converter is being assembled, the disk of the microphone must be in a vertical position, as shown in Fig. 2.

The converter is comparatively insensitive to electrical interference and may be used without screening, even in places where there is a high level of interference. Any pneumatic or pneumohydraulic pick-up of the Marey capsule, rubber cuff, plethysmograph, oncometer or other suitable type may be connected to the pneumatic transmission system. When a pneumatic pick-up is used with a transmission system 80 cm in length the inertia of the converter does not exceed 1 millisec, i.e., for practical purposes the converter can be regarded as having no inertia.

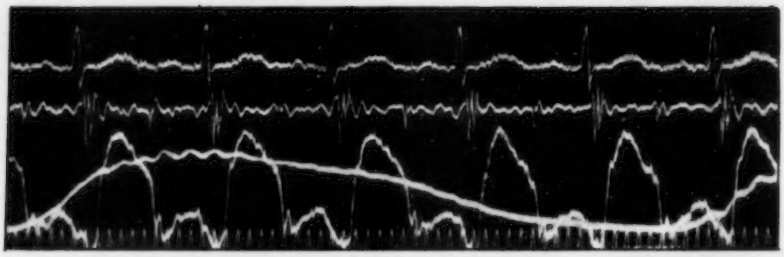


Fig. 3. Specimen of a simultaneous recording of the functions of different organs of a rabbit, using the MPO-2 loop oscillograph and the UIPP-2 amplifier.

Significance of the curves (from above down): electrocardiogram — fourth chest lead CR_4, phonocardiogram, pulsation of the right common carotid artery (recording from the vessel through a fold of skin by means of a simple pneumoelectric converter), respiration (recording by means of a pneumatic cuff and a simple converter), time marker (0.02 second).

In Fig. 3 is given a specimen tracing of the respiration and the pulse in the carotid artery of a rabbit, recorded by means of the pneumoelectric converter.

SUMMARY

The main part of the simple pneumo-electric converter is the carbon button microphone "MK-10", connected according to a special scheme. The converter enables signals received from pneumatic and hydraulic pick-ups, which are employed in physiological investigations, to be recorded on a loop oscillograph.

COLORIMETRIC ESTIMATION OF THIAMIN BY THE NINHYDRIN REACTION

Yu. M. Ostrovskii and M. A. Gvozdeva

From the Vitebsk Medical Institute and the Laboratory of the Regional Hospital

(Received February 23, 1959. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In spite of the high sensitivity of the various modifications of the Jansen [1] thiochrome method of estimation of thiamin, this method is not always suitable because of the necessity of using special apparatus. Many of the colorimetric methods of estimation of thiamin at present known [2] suffer from considerable disadvantages on account of their clumsiness, the fact that large quantities of reagents are required and hence, the time-consuming nature of the analysis itself.

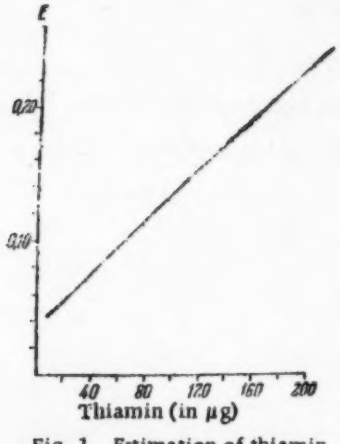


Fig. 1. Estimation of thiamin by the ninhydrin reaction.

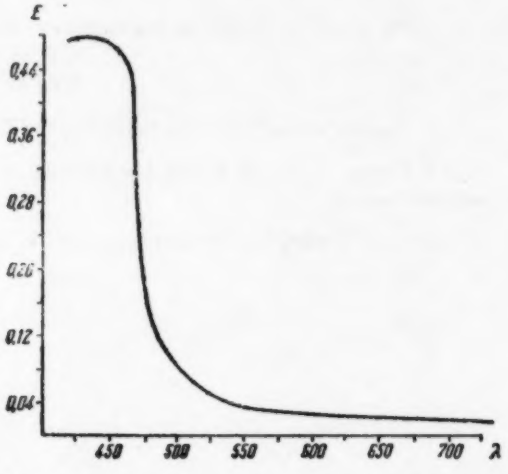


Fig. 2. Spectral characteristics of a sample containing 200 μg of thiamin.

We have developed a new and extremely simple method of estimation of thiamin, based on its reaction with ninhydrin. When thiamin is heated with ninhydrin in a weakly acid medium, a yellow compound is formed which lends itself to quantitative colorimetry. The fact that the concentration of thiamin and the intensity of the color formed are proportional to each other (Fig. 1) over a wide range of concentrations enables this method to be recommended for practical use. With no additional requirements, the method is very suitable for the quantitative testing of pharmaceutical preparations of thiamin. When other substances are to be tested, for example in vitamin assay of food products, the method can be used after preliminary isolation of the thiamin by adsorption on silica-gel or on the Soviet preparation "cationit SDV-3" [3].

Reagents required:

1) M/15 phosphate buffer, pH = 6.8

2) 1% aqueous solution of ninhydrin.

Method of estimation. To 3 ml of the phosphate buffer is added the test solution (preliminarily neutralized, if necessary), containing 10-200 μg thiamin and 0.5 ml of 1% ninhydrin solution. The tube containing the mixture is placed in boiling water for 20 minutes, after which it is cooled and distilled water is added to make the volume up to 5 ml. The yellow color thus formed has maximum absorption in the short-wave region of the visible spectrum. The spectral characteristics of a test sample containing 200 μg of thiamin, taken on the FM photometer, are shown in Fig. 2. The maximum sensitivity is obtained by working with a violet filter. In the absence of a photometer, perfectly satisfactory results may be obtained by working with the light blue filter of the FEK-M photoelectrical colorimeter or with the 7th filter of the KOL-1 visual colorimeter. In the latter case, a suitable quantity of standard thiamin solution is tested simultaneously. Colorimetry can be carried out immediately after cooling, although the color is stable and was shown by confirmatory tests to remain almost unchanged for 24 hours.

The reproducibility of the results is perfectly satisfactory – the values obtained with standard solutions prepared at different times or with fresh buffer solutions or ninhydrin always coincided, with very slight variations (± 2%), with the calibration curve obtained in the first place.

The mechanisms of the reaction has not yet been finally elucidated. It has been shown only that the formation of the colored compound is associated with the thiazole part of the thiamin molecule. The free amino group of the pyrimidine ring of the thiamin probably does not take part in this reaction, for formaldehyde, which blocks the reaction between amino groups and ninhydrin, does not affect the course of the reaction with thiamin.

SUMMARY

The compound formed by the reaction between thiamin and ninhydrin in a weakly acid medium is of use in the colorimetric estimation of the concentration of thiamin in aqueous solution.

The optimal conditions for this reaction were studied and established.

LITERATURE CITED

[1] A. A. Dmitrovskii, Vitamin Resources and Their Utilization, Vol. 3, p. 100. Moscow, 1955 [In Russian].

[2] P. Gyorgy and S. H. Rubin, The Biochemistry and Physiology of Vitamins, Vol. 4, p. 7. Moscow, 1951 [Russian translation].

[3] B. C. P. Jansen, Rec. trav. chim., 1936, v. 55, p. 1046.

A STUDY OF THE PROPERDIN SYSTEM

COMMUNICATION I. A SIMPLIFIED METHOD OF ESTIMATION OF PROPERDIN

K. M. Rozental' and G. B. Savel'vol'f

From the Division of Microbiology (Head — Prof. V. I. Ioffe)
of the Institute of Experimental Medicine of the AMN SSSR, Leningrad

(Received March 21, 1958. Presented by Active Member AMN SSSR
N. N. Anichkov)

The properdin (from the Latin word perdere — to destroy) system was first described by Pillemer and his coworkers [2] in 1954. It is a protein fraction of the serum which, in the authors' opinion, determines the general (nonspecific) resistance of the body to infection, by bringing about lysis of bacteria and inactivation of viruses. This property, and the therapeutic action of properdin in experimental radiation sickness, have attracted considerable attention to this substance.

The action of properdin is shown only in the presence of the other components of the system, namely all four fractions of complement and magnesium ions. The titration of properdin in the serum is based on its power of inactivating the third complement fraction C'_3 in prescribed conditions and in the presence of zymosan (at 37°). It may therefore be estimated by two methods: by the ability of the test serum to lower the bactericidal titer of complement, and by its ability to lower its hemolytic activity. In these experiments the ordinary complement must not be used, but complement from which its own properdin has been removed (the so-called Rp, obtained by keeping complement with a suspension of zymosan for 1 hour at 17°).

Estimation of properdin by the first method, in spite of the apparent lucidity of the scheme of the experiment, in fact proved very complicated. Pillemer therefore considered it necessary to develop and recommend the second method. The essence of this method is as follows. Into a series of test tubes containing diminishing volumes of test serum (from 0.25 to 0.15 ml) are added Rp (0.25 ml by volume), a suspension of zymosan (0.1 ml) and veronal buffer (to 0.75 ml). After standing for 1 hour at 37°, the content of the third fraction of complement in the supernatant fluid of each tube is estimated. For this purpose, to 0.2 ml of supernatant fluid is added R_3 (i.e., complement without third fraction) in a dose of 2 units (in a volume of 0.05 ml), veronal buffer (to 0.5 ml) and hemolytic system (1 ml). The onset of hemolysis demonstrates the presence of C'_3 in the titrated fluid, and absence of hemolysis indicates total inactivation of this fraction of complement. The properdin titer in the test samples is determined by the amount of inactivated C'_3.

Pillemer uses preparations of Rp from human serum and considers that preparations containing not less than 120 units C'_3/ml* are suitable for the experiments.

---

* According to Pillemer, the unit of C'_3 is that quantity which, in the presence of 2 units of R_3, will cause hemolysis in standard experimental conditions (a volume of 1.5 ml, of which 1 ml is hemolytic system). The unit of R_3 is the minimum dose of active complement (before removal of third fraction from it) causing 100% hemolysis in the same standard experimental conditions. The unit of properdin is the amount which will inactivate (in the presence of zymosan) 120 units of third fraction of complement in 1 ml of Rp.

TABLE 1

Scheme of the Experiment for Using Preparations of Rp from Guinea Pig Serum

I

Selection of doses of Rp and C'_3 for Making Up Working Mixtures

Dilution of C'_3	Dilution of Rp					
	1 : 10	1 : 15	1 : 20	1 : 30	1 : 40	1 : 60
1 : 10	-	-	-	-	-	-
1 : 15	-	-	-	-	-	+
1 : 20	-	-	-	-	±	++
1 : 25	-	-	-	-	+	+++
Veronal buffer	-	±	++	+++	++++	++++

II

Main experiment Rp + C'_3 (working mixture . . . . . 0.2 ml
Suspension of zymosan . . . . 0.1 ml
Test serum (in different dilutions) . . . . 0.2 ml
Veronal buffer . . . . 0.1 ml

1 hour at 37°; centrifugation . . . . .
To the supernatant fluid from each tube (0.3 ml) is added 0.2 ml of hemolytic system.

Legend to this and subsequent tables: - hemolysis; +, ++, +++ and ++++ various degrees of inhibition of hemolysis.

He himself points out, however, that such preparations of Rp can be obtained from human sera in not more than 20% of experiments. Nor was he able to obtain satisfactory preparations of R_3 from human sera.

The difficulties which arise in connection with the use of preparations from human serum and with the complexity of the experiment itself, have made it imperative to seek other possible methods: 1) to find an infallible method of obtaining Rp with a higher content of third fraction; 2) to dispense, if possible, with the use of the preparation of Rp and to replace it with crude serum with a low properdin content, which could be ignored, and 3) to simplify the plan of the experiment. This was the subject of our research, the results of which are described below.

EXPERIMENTAL METHOD AND RESULTS

The comparative study of the complement in man and the guinea pig, in respect to their content of the individual components, gave us the idea in the first place, of preparing Rp from a mixture of the corresponding sera, and later, from the serum of the guinea pig alone. In the first case the optimal mixture was one of 4 volumes of guinea pig serum and 1 volume of human serum. The Rp preparations obtained from such a mixture had a much higher hemolytic activity than analogous preparations from human serum. Nevertheless their titer was only half that of the crude mixture from which they were obtained. The same thing was observed in relation to preparations of Rp obtained directly from guinea pig's complement. We therefore added to the preparation of Rp a small quantity of C'_3 in the form of complement heated to 52°. We usually had to add 1 part of C'_3 to 2 parts of Rp. This completely restored the hemolytic activity of the preparation.

The properdin of the test sera was estimated as follows. As a preliminary step the optimal concentrations of Rp and C'_3 were titrated, as shown in Table 1. We selected a dilution of Rp which itself would give almost total hemolysis (in this particular case 1:12), and C'_3 was added in a concentration sufficient to increase the Rp activity two- or threefold (in this particular experiment 1:16). To 0.2 ml of the preparation was added 0.2 ml of

TABLE 2

Inactivation of Guinea Pig Complement by Rat Serum in the Presence of Zymosan

Dilution of rat serum	Dilution of complement			Control (addition of rat serum without zymosan to 1:30 complement)
	1:20	1:25	1:30	
1:100	++++	++++	++++	—
1:150	++++	++++	++++	—
1:200	++++	++++	++++	—
1:300	−+	−+++	+++	—
1:400	—	+	−+++	—
1:600		—	−+−	
Veronal buffer	—	—	—	—

the test serum in different dilutions, 0.1 ml of a suspension of zymosan* and 0.1 ml of veronal buffer.** After allowing the mixture to react at 37°, the contents of the tube were centrifuged, 0.3 ml of the supernatant fluid was withdrawn and to it was added 0.2 ml of hemolytic system. The result of the test was read after 20 minutes at 37°. Absence of hemolysis showed inactivation of the C'_3. The properdin content could be judged by the minimum dose of test serum in which hemolysis was observed to be inhibited.

For all its simplicity, however, this method had certain essential drawbacks: the Rp preparation cannot be preserved under ordinary conditions but must be obtained ex tempore; titration has to be carried out immediately, followed by the main experiment. This was very time consuming and very laborious. We therefore tried to do without a special preparation of Rp, by substituting guinea pig complement untreated in any way.

The properdin content in guinea pig complement is in fact negligible, and after dilution it is still further reduced, so that this amount of properdin can be ignored. At the same time, guinea pig complement has characteristically high values of C'_3 and other fractions.

The suitability of crude guinea pig complement to replace the Rp preparation was tested in experiments in which rat serum was used as a source of properdin. We chose this serum because, as reported in the literature, it is distinguished by a high properdin content which, in ordinary conditions, does not undergo any very considerable variation.

Table 2 shows the results of one of the experiments in which we tested the ability of rat serum to inactivate complement in the presence of zymosan.

Rat serum was used in dilutions from 1:100 to 1:600 and was added in equal volumes (0.2 ml) to complement, diluted from 1:20 to 1:30. To each tube was added a suspension of zymosan in a volume of 0.1 ml (containing 0.5 mg of the preparation) and veronal buffer (0.1 ml). The mixture was kept for 1 hour at 37°. Veronal buffer was added to the control tubes instead of rat serum. A second control series was also used in which complement, diluted 1:30, was mixed with the above doses of rat serum, but veronal buffer was added instead of the sus-

* Zymosan was prepared by Pillemer's formula [2] from yeast of strain No. 7 from the Leningrad yeast factory. The working dose of each batch of zymosan was titrated and the dose chosen which gave the most active Rp preparation by treatment of complement. In these experiments we used a suspension containing 0.5 mg zymosan in 0.1 ml.

** Composition of the veronal buffer: sodium chloride 8.5 g, veronal 0.575 g, medinal 0.375 g, magnesium chloride 0.5 ml, calcium chloride (M/1) 0.15 ml, distilled water to 1 liter.

TABLE 3

Comparative Evaluation of the Properdin Content of Different Sera (Typical Experimemt)

Concentration of test sera (in %)	Rat serum (initial concentration 1:200)		Donor's serum		Sera from patients			
					1		2	
			initial concentration 1:20					
	experiment	control (without zymosan)	experiment	control (without zymosan)	experiment	control (without zymosan)	experiment	control (without zymosan)
100	++++	—	++++	—	++++	—	++	—
90	++++	—	++++	—	++++	—	++	—
80	++++	—	++++	—	+++	—	—	—
70	++++	—	++++	—	++	—	—	—
60	++++	—	++++	—	—	—	—	—
50	++++	—	++++	—	—	—	—	—
40	++++	—	++++	—	—	—	—	—
33	+++	—	+++	—	—	—	—	—
25	+	—	++	—	—	—	—	—
20	—	—	+	—	—	—	—	—
Control (complement, zymosan, buffer)	—	—	—	—	—	—	—	—

pension of zymosan. After all the tubes had been kept for 1 hour at 37°, 0.3 ml was withdrawn from each, and to it was added 0.2 ml of hemolytic system; all the tubes were then placed in a water bath at 37°. The results of the experiment were read when complete hemolysis took place in the control tubes. Complement, when treated with small doses of rat serum in the presence of zymosan, lost its hemolytic activity. At the same time, the addition of rat serum itself, without zymosan, did not affect the complement titer, nor did treatment with zymosan in the presence of buffer. Rat serum, inactivated by heat, also had no action whatsoever in the presence of zymosan, for it was deprived of its properdin. It might be mentioned that, with a higher concentration of complement, a larger dose of rat serum was required for its inactivation. It was therefore necessary to titrate the optimal dilution of complement for the main experiment. In accordance with the results shown in Table 2, this dilution must be considered to be 1:27—1:30, for this dose itself produced complete hemolysis and, at the same time, when treated with small doses of rat serum in the presence of zymosan, it lost not less than half its activity.

Some doubt may arise whether a sufficient quantity of all 4 fractions required for manifestation of the action of properdin is present in the dilution of complement chosen. This was tested as follows. An experiment was carried out on one series of tubes in the ordinary way, and in another series complement was not diluted with buffer but with a preparation of R_3. In the second case, therefore, the amount of the first, second and fourth fractions of complement was greatly increased. It was found that the action of the properdin of the rat serum was the same in both experiments. From this it follows that all the components of complement were present in the first series too, in sufficient quantity to demonstrate the action of properdin.

The results obtained enabled the properdin content of different sera to be compared. An example of such an experiment is given in Table 3.

A rat serum, a healthy donor's serum and two sera from patients were tested simultaneously. The initial dilution of the rat serum was 1:200 and of the others 1:20. In each successive dilution the concentration of the test serum diminished by 10%. The experiment was carried out by the scheme described above. From each dilution of the test sera 0.2 ml was poured into a test tube, and to it was added an equal volume of complement in a titrated dilution (1:25) and 0.1 ml each of a suspension of zymosan and veronal buffer. The necessary controls were set up at the same time. The tubes were kept for 1 hour at 37° and centrifuged; to 0.3 ml of the supernatant fluid was added 0.2 ml of hemolytic system and the mixture was kept on a water bath at 37° until the onset of hemolysis in the controls.

It can be seen from Table 3 that 50% hemolysis (inhibition of hemolysis ++) developed if the rat serum was in dilutions of 1:600-1:700 (>25%<33% of the original dilution). The donor's serum showed the same action in a dilution of 1:80, the first patient's serum in a dilution of 1:33 and the second — 1:20. This gave grounds for considering that the properdin content of the donor's serum was one-eighth that of the rat serum, that of the first patient one-twelfth to one-fourteenth, and that of the second patient one-thirtieth that of the rat serum.

It was thus possible to express the properdin content as quantitative values by comparing the action of the test serum with that of a standard properdin preparation. We considered that rat serum fulfilled this purpose. According to Pillemer, rat sera contain 25-30 units of properdin per ml. The method described enabled the conventional unit of properdin to be considerably reduced. Since the final effective dilution of rat serum was 1:600, it could be accepted that 1 ml contains 600 conventional units of properdin. In accordance with this definition, the properdin content was calculated in the test sera. The establishment of a smaller unit of properdin widened the range of titration, which had definite advantages.

It must be pointed out that the properdin titer is maintained for a sufficiently long time in rats serum if kept in a frozen state (-20°). It is therefore desirable to use as a standard a mixture of several rat sera, kept under the above conditions. In order to obtain comparable results, each new mixture must be titrated in the same experiment with the previous mixture. It is also possible to use the same complement for 7-10 days, if it is kept in the frozen state. The method described enabled a considerable number of sera from healthy persons and patients and also from experimental animals to be investigated without undue difficulty. The results of these investigations will be described in the next communication.

SUMMARY

This work deals with an experimental analysis of the two possible methods of properdin determination and their disadvantages. The authors suggest a new simple method which consists of the substitution of Rp by a guinea pig complement with the utilization of normal rat serum (characterized by high and stable properdin titer) as a standard. The optimal dilution of the complement, which loses its hemolytic activity after treatment with rat serum in 1:600 — 1:800 dilution in presence of zymosan, is determined in a preliminary experiment. The amount of the serum under investigation capable of giving the same result with the titrated dose of complement, is determined in the main experiment.

LITERATURE CITED

[1] L. Pillemer, Transactions N. Y. Ac. Sc., 1955, ser. 2, v. 17, p. 526.

[2] L. Pillemer et al., Science, 1954, v. 120, p. 279-285.

[3] L. Pillemer, L. Blum and J. Pensky, er al., J. Immunol., 1953, v. 71, p. 331-338.

[4] L. Pillemer, I. H. Lepow and L. Blum., J. Immunol. 339-345.

[5] L. Pillemer, O. D. Rattnoff and L. Blum et al., J. Exper. Med., 1953, v. 97, p. 573-589.

[6] L. Pillemer, L. Blum and I. H. Lepow et al., J. Exper. Med. 1956, v.103, p.1-15.

[7] A. Wardlaw and L. Pillemer., J. Exper. Med. 1956, p. 553-574.

* In Russian.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for Experimental Morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

For the first time in English . . . .

RESEARCH ON NOVOCAIN THERAPY IN OLD AGE

American researchers and physicians can now judge for themselves the significance of the dramatic results of novocain therapy in maladies of old age, as reported by Dr. Anna Aslan, *Director of the Institute of Geriatrics, Bucharest, Rumania,* and her co-workers.

These seven papers, now in complete English translation, originally appeared in the West German journal *"Die Therapiewoche."* The authors report the results of large-scale clinical application of novocain injections for treatment of the sequelae of old age. The research has continued over a seven-year period and has covered more than 5,000 people. Dr. Aslan and her colleagues claim to have achieved a reversal of phenomena previously considered irreversible, e.g., in *cerebral arteriosclerosis;* they report sustained improvement in senile Parkinsonism, ichthyosis, and senile keratosis, and a reduction in arteriosclerotic symptoms.

These reports have been translated and published in English so that they can be more readily subjected to objective analysis and evaluation by qualified scientists and physicians.

TITLES OF REPORTS

A New Method for Prophylaxis and Treatment of Aging with Novocain—Eutrophic and Rejuvenating Effects • Prevention and Treatment of Diseases of the Aged—Views Concerning Treatment of Old Age • Biochemical and Physiological Criteria of Aging and Effects Thereon by Treatment, as a Measure of Rehabilitation • Recent Experiences on the Rejuvenating Effect of Novocain Together with Experimental, Clinical, and Statistical Proof • Results of Treatment with Novocain in Dysmetabolic Arthropathies • Significance of Biomorphosis for Aging, Disease and Rejuvenation • First Experiences with Novocain Therapy of Old People.

The Complete Collection $12.50

CONSULTANTS BUREAU, INC.

227 WEST 17TH STREET, NEW YORK 11, N. Y

ENGLISH TRANSLATIONS OF RUSSIAN BIOLOGICAL JOURNALS

The American Institute of Biological Sciences, under a grant from the National Science Foundation, is currently translating and publishing seven Russian research journals in biology.

DOKLADY: BIOLOGICAL SCIENCES SECTIONS

6 issues per year. Subscriptions: $20.00 per year, U.S.A. & Canada
15.00 per year, university and nonprofit libraries
2.50 additional to each price, foreign
4.00 each, single copies

DOKLADY: BOTANICAL SCIENCES SECTIONS

6 issues per year. Subscriptions: $7.50 per year, U.S.A. & Canada
5.75 per year, university and nonprofit libraries
1.50 additional to each price, foreign
1.50 each, single copies

DOKLADY: BIOCHEMISTRY SECTION

6 issues per year. Subscriptions: $15.00 per year, U.S.A. & Canada
10.00 per year, university and nonprofit libraries
3.00 additional to each price, foreign
4.50 each, single copies

MICROBIOLOGY • MIKROBIOLOGIYA

6 issues per year. Subscriptions: $20.00 per year, U.S.A. & Canada
15.00 per year, university and nonprofit libraries
2.50 additional to each price, foreign
4.00 each, single copies

PLANT PHYSIOLOGY • FIZIOLOGIYA RASTENII

6 issues per year. Subscriptions: $15.00 per year, U.S.A. & Canada
11.00 per year, university and nonprofit libraries
2.00 additional to each price, foreign
3.00 each, single copies

ENTOMOLOGICAL REVIEW • ÉNTOMOLOGICHESKOE OBOZRENIE

4 issues per year. Subscriptions: $25.00 per year, U.S.A. & Canada
12.00 per year, university and nonprofit libraries
3.00 additional to each price, foreign
7.50 each, single copies

SOVIET SOIL SCIENCE • POCHVOVEDENIYE

12 issues per year. Subscriptions: $40.00 per year, U.S.A. & Canada
20.00 per year, university and nonprofit libraries
3.00 additional to each price, foreign
4.50 each, single copies

I wish to subscribe to:

- ☐ DOKLADY: BIOLOGICAL SCIENCE SECTIONS
- ☐ DOKLADY: BOTANICAL SCIENCE SECTIONS
- ☐ DOKLADY: BIOCHEMISTRY SECTION
- ☐ PLANT PHYSIOLOGY
- ☐ MICROBIOLOGY
- ☐ ENTOMOLOGICAL REVIEW
- ☐ SOVIET SOIL SCIENCE

Enclosed: (Check or Money Order) _____ CB

NAME: _____

ADDRESS: _____

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES
2000 P St., N. W. Washington 6, D. C.